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(54) Title: COMPOUNDS HAVING MIF ANTAGONIST ACTIVITY

(57) Abstract: There is disclosed a genus of optionally substituted Schiff base condensation products (and the carba analogs thereof) comprising an amino acid component and a benzaldehyde component, that have MIF (macrophage migration inhibitory factor) antagonist activity. The compounds are useful for treating a variety of diseases involving inflammatory activity or pro-inflammatory cytokine responses, such as autoimmune diseases, asthma, arthritis, EAE, ARDS and various forms of sepsis and septic shock, and other conditions characterized by underlying MIF responses including, for instance, tumor growth and neovascularization.

COMPOUNDS HAVING MIF ANTAGONIST ACTIVITY

Cross Reference to Related Applications

5   This application claims the benefit of U.S. Provisional Application No. 60/162,467, filed October 29, 1999, hereby incorporated by reference.

Technical Field of the Invention

The present invention provides a genus of optionally substituted Schiff base
10   condensation products (and the carba analogs thereof) comprising an amino acid component and a benzaldehyde component, that have MIF (macrophage migration inhibitory factor) antagonist activity. Specifically, the compounds are useful for treating a variety of diseases involving inflammatory activity or pro-inflammatory cytokine responses, such as autoimmune diseases, asthma, arthritis, EAE, ARDS and various forms of sepsis and septic shock, and other
15   conditions characterized by underlying MIF responses including, for instance, tumor growth and neovascularization.

Background of the Invention

Human MIF was first cloned in 1989 and its activity has been investigated in a number
20   of studies. MIF was the first lymphokine to be discovered and was originally identified by its ability to prevent the migration of guinea pig macrophages *in vitro* (Bloom & Bennett, *Science* 153:80-82, 1966; David, *Proc. Natl. Acad. Sci. USA* 56:72-77, 1966). Given this activity, the role of MIF activity in inflammation and the immune system was investigated, however the precise role of MIF in either local or systemic inflammatory responses remained largely
25   undefined in the course of this early work. Likewise the role of MIF in other physiological and pathophysiological is still being defined.

MIF was reported to be associated with delayed-type hypersensitivity reactions (Bloom & Bennett, 1966, *supra*; David, 1966, *supra*), to be produced by lectin-activated T-cells (Weiser et al., *J. Immunol.* 126:1958-1962, 1981), and to enhance macrophage adherence,
30   phagocytosis and tumoricidal activity (Nathan et al., *J. Exp. Med.* 137:275-288, 1973; Nathan et al., *J. Exp. Med.* 133:1356-1376, 1971; Churchill et al., *J. Immunol.* 115:781-785, 1975). Unfortunately, many of these early studies used mixed culture supernatants that were shown later to contain other cytokines, such as IFN-γ and IL-4, that also have macrophage migration inhibitory activity (McInnes & Rennick, *J. Exp. Med.* 167:598-611, 1988; Thurman et al., *J.*
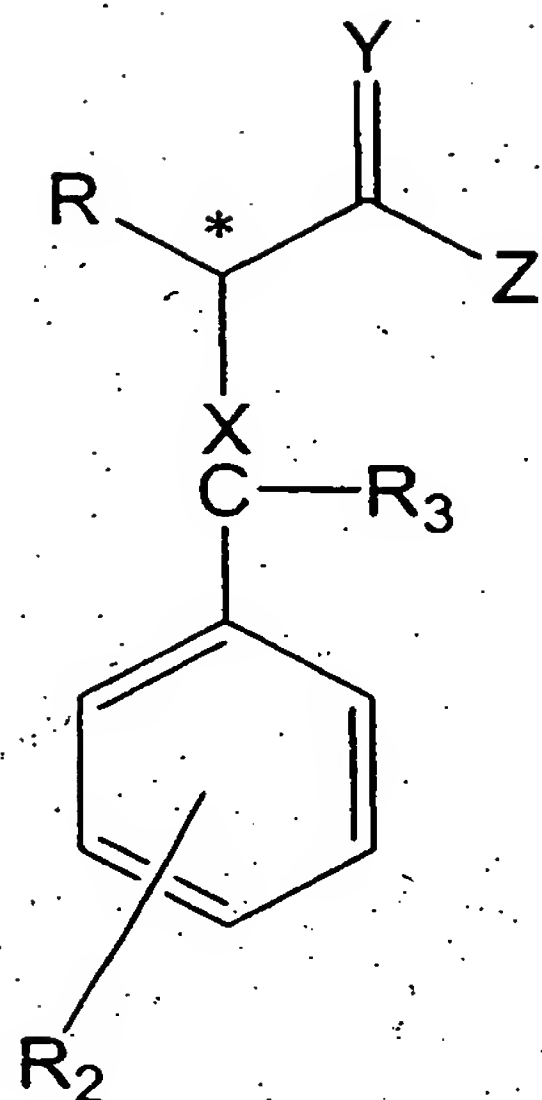35   *Immunol.* 134:305-309, 1985).

Recombinant human MIF was originally cloned from a human T cell library (Weiser et al., *Proc. Natl. Acad. Sci. USA* 86: 7522-7526, 1989), and was shown to activate blood-derived macrophages to kill intracellular parasites and tumor cells *in vitro*, to stimulate IL-1β and TNFα expression, and to induce nitric oxide synthesis (Weiser et al., *J. Immunol.* 147:2006-

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2011, 1991; Pozzi et al., *Cellular Immunol.* 145:372-379, 1992; Weiser et al., *Proc. Natl. Acad. Sci. USA* 89:8049-8052, 1992; Cunha et al., *J. Immunol.* 150:1908-1912, 1993). While the conclusions available from several of these early reports are confounded by the presence of a bioactive mitogenic contaminant in the recombinant MIF preparations used, the potent pro-

5   inflammatory activities of MIF have been confirmed in other studies that do not suffer from this complicating factor (reviewed in Bucala, *The FASEB Journal* 10:1607-1613, 1996). More recent MIF studies have capitalized on the production of recombinant MIF in purified form as well as the development of MIF-specific polyclonal and monoclonal antibodies to establish the biological role of MIF in a variety of normal homeostatic and pathophysiological settings

10   (reviewed, for instance, in Rice et al., *Annual Reports in Medicinal Chemistry* 33:243-252, 1998). Among the most important insights of these later reports following the "re-discovery" of MIF has been the recognition that MIF not only is a cytokine product of the immune system, but also is a hormone-like product of the endocrine system, particularly the pituitary gland. Moreover, this recent work has underscored the potent activity of MIF as a counter-regulator

15   of the anti-inflammatory effects of the glucocorticoids (both those endogenously released and those therapeutically administered), with the effect that the normal activities of glucocorticoids to limit and suppress the severity of inflammatory responses are inhibited by MIF, such that the endogenous MIF response is seen as a cause or an exacerbative factor in a variety of inflammatory diseases and conditions (reviewed in Donnelly and Bucala, *Molecular Medicine*

20   *Today* 3:502-507, 1997). MIF has also been linked to tumor growth and neovascularization (angiogenesis), suggesting a further need for MIF antagonists in the area of oncology and cancer treatment (Chesney et al., *Molecular Medicine* 5:181-191, 1999).

　　　　This experimental work underscores the need for inhibitors of MIF, and MIF antagonists have been identified and shown to have a variety of therapeutic activities, including

25   activity for multiple inflammatory diseases, cytokine-mediated toxicities, asthma, and autoimmune diseases (*e.g.*, rheumatoid arthritis, graft versus host disease, insulin-dependent diabetes, and various forms of lupus). These MIF antagonist agents, however, have been "biological" agents that are designed to bind MIF (*e.g.*, antibody) and to prevent its expression (*e.g.*, antisense oligonucleotide). Such biological agents, unfortunately, have certain

30   limitations with regard to their clinical utility. Therefore, there is a need in the art to discover and develop small organic molecules that function as MIF antagonists and further posses the benefits of small organic molecule therapeutics versus larger, polymeric protein and nucleic acid-based-based therapeutic agents.

35   **Summary of the Invention**
　　　　The present invention provides a compound of Formula I:

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wherein X is nitrogen or carbon;

wherein Y is O, $C(R_1)_2$, or S;

10   wherein Z is OH, R_1, $-CH_2-N(R_1)_2$, or SR_1;

wherein R_1 is independently H, straight or branched C_1-C_6 alkyl, straight or branched C_2-C_6 alkenyl, or if R_1 is attached to carbon (but not if R_1 is attached to N or S), straight or branched C_1-C_6 alkoxy;

wherein R_2 is a single or multiple substitution independently H, OH, R_1, $N(R_1)_2$, SR_1 or

15   a halogen;

wherein R_3 is absent, H, R_1, or a halogen;

wherein * and C mark potentially asymmetric carbon atoms and, in each instance of R, X, Y, Z, R_1, and R_3 where * or C or both are asymmetric, all diastereomers are included in Formula I;
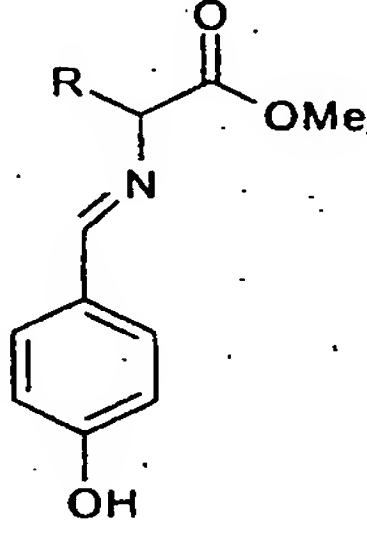
20   wherein R is the side-chain of any naturally occurring alpha amino acid such that R together with X, Y and Z comprise, when Y is O and Z is OH, an aromatic, aliphatic or heterocyclic D or L alpha amino acid moiety linked as a Schiff base or a reduced Schiff base (if X is N) or as a carba analog thereof (if X is C) to an optionally substituted benzaldehyde moiety, and wherein if Y is other than O, or Z is other than OH, R together with X, Y and Z

25   comprise a substituted amino acid moiety, an analog of an amino acid moiety or a protected amino acid moiety;

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and pharmaceutically acceptable salts thereof.

Preferably, X is N, Y is O, and Z is OMe such that together with R they form a protected L aliphatic or aromatic amino acid moiety. Preferably, R_2 is OH, and R_3 is H. Even more preferably R_2 is in a para position to comprise a para-hydroxybenzaldehyde moiety.

5   A preferred subset of these preferred compounds is described by Formula II, wherein R is H (compound 1); wherein R is $-CH_3$ (compound 2 or compound 2a corresponding to L alanine and compound 2b corresponding to D alanine); wherein R is $-CH(CH_3)_2$ (compound 3); wherein R is $-CH_2CH(CH_3)_2$ (compound 4); wherein R is $-CH(CH_3)CH_2CH_3$ (compound 5); wherein R is $-CH_2CH_2SCH_3$ (compound 6); and wherein R is $-CH_2OH$ (compound 7).

10   Formula II:

		Inhibition (IC_{50})
R=H	1	>100μM
R= CH_3	2a	80μM
R= CH_3	2b	70μM
R= $CH(CH_3)_2$	3	>100μM
R=$CH_2CH(CH_3)_2$	4	
R=$CH(CH_3)CH_2CH_3$	5	
R=$CH_2CH_2SCH_3$	6	100μM
R=CH_2OH	7	100μM

Still more preferred, R is the side-chain of a naturally-occurring aromatic alpha amino acid, X is N, Y is O, and Z is OMe such that together with R they form a protected L aromatic amino acid moiety. Most preferably, R_2 is OH, and R_3 is H. Most preferably, R_2 is in a para
15   position to comprise a para-hydroxybenzaldehyde moiety product. Most preferably, R is $-CH_2$-phenyl (compound 8), or R is $-CH_2$-para-hydroxyphenyl (compound 9), or R is $-CH_2$-indoyl (compound 10).

The invention further provides a pharmaceutical composition comprising a compound of Formula I, as a pharmaceutically acceptable salt thereof, together with a pharmaceutically
20   acceptable carrier or diluant.

The invention further provides a compound of formula I, or a pharmaceutically acceptable salt thereof, as a pharmaceutical composition comprising either of the aforesaid, for use in a medicine or for the manufacture of a medicament for the treatment or prevention of cancer or of inflammatory disorders including arthritis, proliferative vascular disease, ARDS
25   (acute respiratory distress syndrome), cytokine-mediated toxicity, sepsis, septic shock,

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psoriasis, interleukin-2 toxicity, asthma and MIF-mediated conditions. The present invention further provides a method for treating or preventing cancer, arthritis, proliferative vascular disease, ARDS (acute respiratory distress syndrome), cytokine-mediated toxicity, sepsis, septic shock, psoriasis, interleukin-2 toxicity, asthma, MIF-mediated conditions, autoimmune

5   disorders including rheumatoid arthritis, insulin-dependent diabetes, multiple sclerosis, graft versus host disease, lupus syndromes, or any condition characterized by local or systemic MIF release or synthesis.

In accordance with the activity of MIF to interfere with the anti-inflammatory effects of steroids (such as the anti-inflammatory glucocorticoids), the compounds of Formula I find

10   further utility to enhance the activity and therapeutic benefits of both endogenously arising and exogenously administered steroidal anti-inflammatory agents. Such benefits may, in some cases, be most evident by a reduced need for steroid therapy (e.g., lower dose amount or frequency; less potent agent; reduced need for systemic administration) or by reduced side-effects associated with steroid administration. The benefits of administering an MIF antagonist

15   agent (and specifically a compound of Formula I) may be realized as a monotherapy, using only the MIF antagonist agent of the present invention, or as a combination therapy with additional anti-inflammatory agents, including especially, but without limitation, an anti-inflammatory steroid. Such combination therapy may be achieved through administration of a single formulation or pharmaceutical composition that combines the MIF antagonist agent

20   (particularly an agent of Formula I) with at least one other anti-inflammatory agent (which may be a steroidal or a non-steroidal anti-inflammatory agent), or through administration of separate formulations or pharmaceutical compositions in conjunction with each other (e.g., one composition comprising the MIF antagonist agent of Formula I, and at least one other composition comprising at least one steroidal or non-steroidal anti-inflammatory agent), or

25   both.

Brief Description of the Drawings

Figure 1 shows that MIF antagonist agents of the presnet invention can suppress serum-

30   induced cell proliferation to a degree equal to the suppressing activity of a neutralizing anti-MIF monoclonal antibody. Figure 1 provides a comparison of the activity of an anti-MIF monoclonal antibody (100 μg/ml) vs L-tryptophan/para-hydroxybenzaldehyde Schiff base (compound 10; 10 μM) vs D-tryptophan/para-hydroxybenzaldehyde Schiff base (compound 10b; 10 μM) to inhibit serum-induced cell proliferaiton in assay populations of quiescent

35   NIH/3T3 fibroblasts. The proliferaiton effect depends on MIF bioactivity, such that neutralization of MIF is reflected in an inhibition of proliferation, and this inhibitory effect is predictive of clinical utility of the active MIF antagonist agent.

Figure 2 compares the activity of MIF antagonist agents of the present invention (and more particularly compounds according to Formula 1) to neutralize the MIF-mediated

protection from apoptosis induced in cultured murine embryonic fibroblasts, which apoptotic response (cell death) is initiated by withdrawal of serum. In this assay, withdrawal of serum initiates an apoptotic response, but the cells can be rescued by addition of recombinant MIF (rMIF) to the culture medium. Treatment with MIF antagonist compound **10** (L-

5   tryptophan/para-hydroxybenzaldehyde Schiff base), but not compound **10b** (D-tryptophan/para-hydroxybenzaldehyde Schiff base), was as effective as a monoclonal anti-MIF antibody treatment at neutralizing rMIF added to the cultures, as evidenced by persistence of apoptosis despite addition of rMIF in cultures treated with compound **10**. Note the dose-related effect of treatment. This antagonistic effect on MIF bioactivity *in vitro* is predictive of

10   clinical utility of the active MIF antagonist agent.

Detailed Description of the Invention

MIF was found to catalyze a tautomerization reaction (Rosengren, et al., *Molecular*

15   *Medicine* 2:143-149, 1996), and although the most active substrate identified to date is the non-physiological D-isomer of dopachrome, the observation that MIF can catalyze a tautomerization reaction points to the possibility of developing inhibitors of MIF enzymatic activities as therapeutic MIF antagonists (see US Patent Application Serial Number 08/602,929, filed 16 February 1996, the disclosure of which is incorporated by reference

20   herein).

According to the present invention, unless otherwise indicated, alkyl groups having three or more carbon atoms may be straight or branched chain. In addition, alkenyl or alkynyl groups having four or more carbon atoms, or alkoxy groups having three carbon atoms, may be straight or branched chain. The compounds of formula I may contain one or more asymmetric

25   centers and can exist as alternative tautomer forms or diastereoisomers. The present invention includes both mixtures and separate individual isomers and tautomers, although, structure-activity data indicates that for some compounds of the inventive genus, one enantiomer has more therapeutic activity than the other.

A class of compounds has been synthesized and further characterized as to the chemical

30   nature of the amino acid moiety. Compounds of one subset are Schiff bases comprising a protected aliphatic amino acid. These include compounds 1-7.

		Inhibition (IC_{50})
R=H	1	>100μM
R= CH_3	2a	80μM
R= CH_3	2b	70μM
R= $CH(CH_3)_2$	3	>100μM
R=$CH_2CH(CH_3)_2$	4	
R=$CH(CH_3)CH_2CH_3$	5	
R=$CH_2CH_2SCH_3$	6	100μM
R=CH_2OH	7	100μM

The present invention encompasses compounds and the use of compounds that are identified as MIF antagonists by their inhibition of an *in vitro* enzymatic assay in which MIF catalyzes the tautomerization of a dopachrome-related MIF substrate to a colorless product. This assay for inhibition of MIF tautomerase activity is predictive of clinical utility as an MIF antagonist therapeutic agent. In the table above (describing compounds 1-7 according to Formula II), an IC_{50} value was obtained for compounds 1-3 and 6-7, as shown in the table, in such an MIF tautomerase assay. Unless specifically indicated to the contrary, references made herein to an inhibitory concentration (*e.g.*, IC_{50} or other activity index) refer to the inhibitory activity of a test compound in an MIF tautomerase assay (as further described in detail, for instance, in Bendrat et al., *Biochemistry* 36:15356-15362, 1997).

The general method of performing an assay for MIF dopachrome tautomerase activity begins with the preparation and oxidation of a DOPA-related substrate precursor, such as L-3,4-dihydroxyphenylalanine methyl ester. This oxidation, typically with sodium periodate, generates the corresponding dopachrome derivative (*e.g.*, L-3,5-dihydro-6-hydroxy-5-oxo-2H-indole-2-carboxylic acid methyl ester), which is typically orange-colored and comprises a convenient substrate for use in a photometric assay for the activity of MIF as a tautomerase. Upon addition of MIF (typically a purified preparation of recombinant MIF at a final concentration of 50-1000 ng/ml), the tautomerization of the colored dopachrome substrate to (in this example) a colorless 5,6-dihydroxyindole-2-caboxylic acid methyl ester product occurs. The enzymatic activity of MIF is measured as the rate of de-colorization of the colored solution of the dopachrome-related substrate in a suitable buffer, typically monitored at 475 nm (or 550 nm for substrate concentrations in excess 0.5 nM). A test compound may be included in the assay solution such that the effect of the test compound on MIF tautomerase activity

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(*i.e.*, as an inhibitor) may be measured by noting the change in kinetics compared to control assays performed in the absence of the test inhibitor compound. In particular, the MIF tautomerase assay may be conducted essentially as follows:

L-3,4-dihydroxyphenylalanine methyl ester (*e.g.*, Sigma D-1507), the dopachrome substrate precursor, is prepared as a 4 mM solution in dd H_2O. Sodium periodate is prepared as an 8 mM solution in ddH_2O. Assay Buffer (50 mM potassium phosphate/1 mM EDTA, pH 6.0) is prepared. Purified recombinant MIF is prepared in 150 mM NaCl/20 mM Tris buffer (pH 7.4) as a stock solution convenient to supply MIF at a final concentration of about 700 ng/ml. Immediately prior to initiating the assay, 3.6 ml dopachrome substrate precursor solution, 2.4 ml periodate solution and 4.0 ml Assay Buffer are combined into a homogeneous mixture (this preparation of dopachrome substrate is suitable for assay use after 1 min and for about 30 min thereafter). Test compound (typically prepared as a concentrated stock in DMSO) and MIF are then combined with 0.7 ml Assay Buffer plus 0.3 ml dopachrome substrate solution to provide the desired final concentration of the test compound in a homogeneous mixture, and the optical density of this assay mixture is monitored at 475 nm. Typically, OD_{475} is recorded every 5-sec for 0-60 sec, and the OD_{475} for a given time point is compared to parallel assays where MIF is not added or the test compound is omitted. Inhibition of MIF tautomerase activity by the test compounds is determined by inhibition of the de-colorization of the assay mixture, typically at the 20 sec time point. IC_{50} values for compounds with MIF tautomerase inhibitory activity, corresponding to the concentration of inhibitor that would inhibit MIF tautomerase activity by 50%, are determined by interpolation of the results from MIF tautomerase assays at several different inhibitor concentrations. These IC_{50} values are taken as a useful predictor of the clinical MIF inhibitory activity of the test compounds.
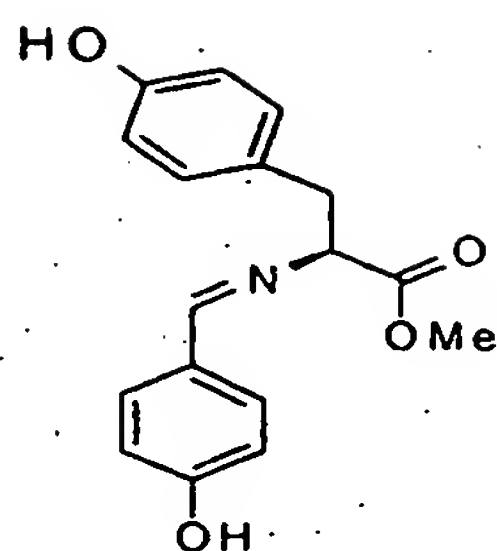
A second exemplary subset of preferred compounds is compounds 8-10 (below), that are Schiff bases of protected L aromatic amino acids. Compound 10 exhibited the most potent MIF antagonist activity with an IC_{50} of 5 μM, while compound 9 had 10 μM IC_{50} and compound 8 showed an IC_{50} of 50 μM in the aforementioned tautomerase assay.
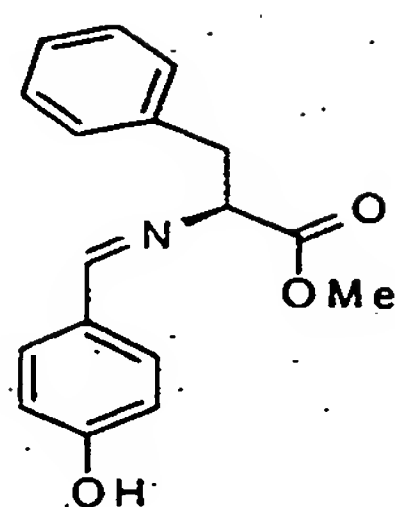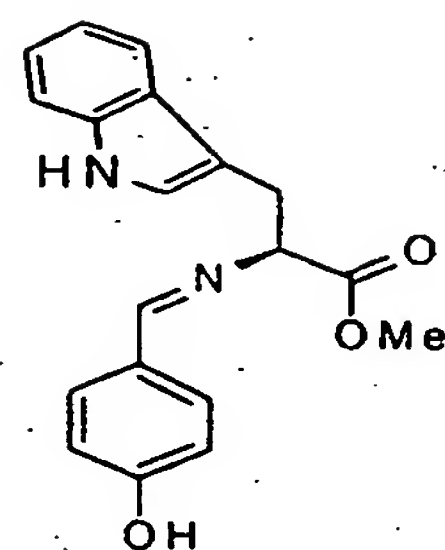
8
IC₅₀   50μM

9
10μM

10
5μM

Two corresponding structures made from D aromatic amino acids to produce the S
enantiomers are compounds 8b and 10b and they show significantly less MIF antagonist
5   activity.
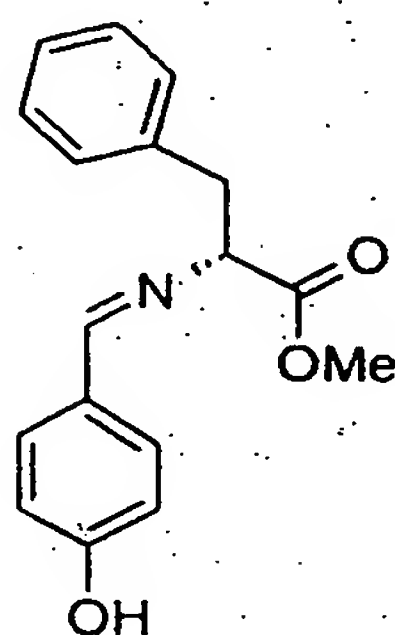
8b
IC₅₀   100μM
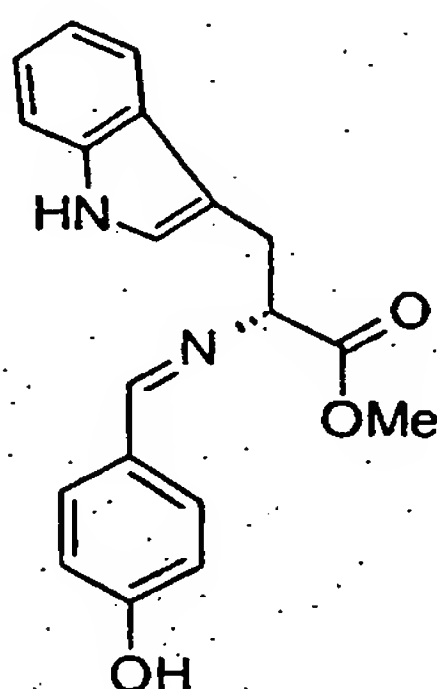
10b
90μM

Compounds 11-13 are reduced products of the aromatic R enantiomers of the amino
acid/para-hydroxybenzyaldehyde Schiff base compounds 8, 9 and 10, as illustrated below
10   (where "N.A." indicates that the compound was not active as an inhibitor of MIF tautomerase
activity at concentrations up to 100 μM).

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11   N.A
12   N.A
13   N.A
IC$_{50}$
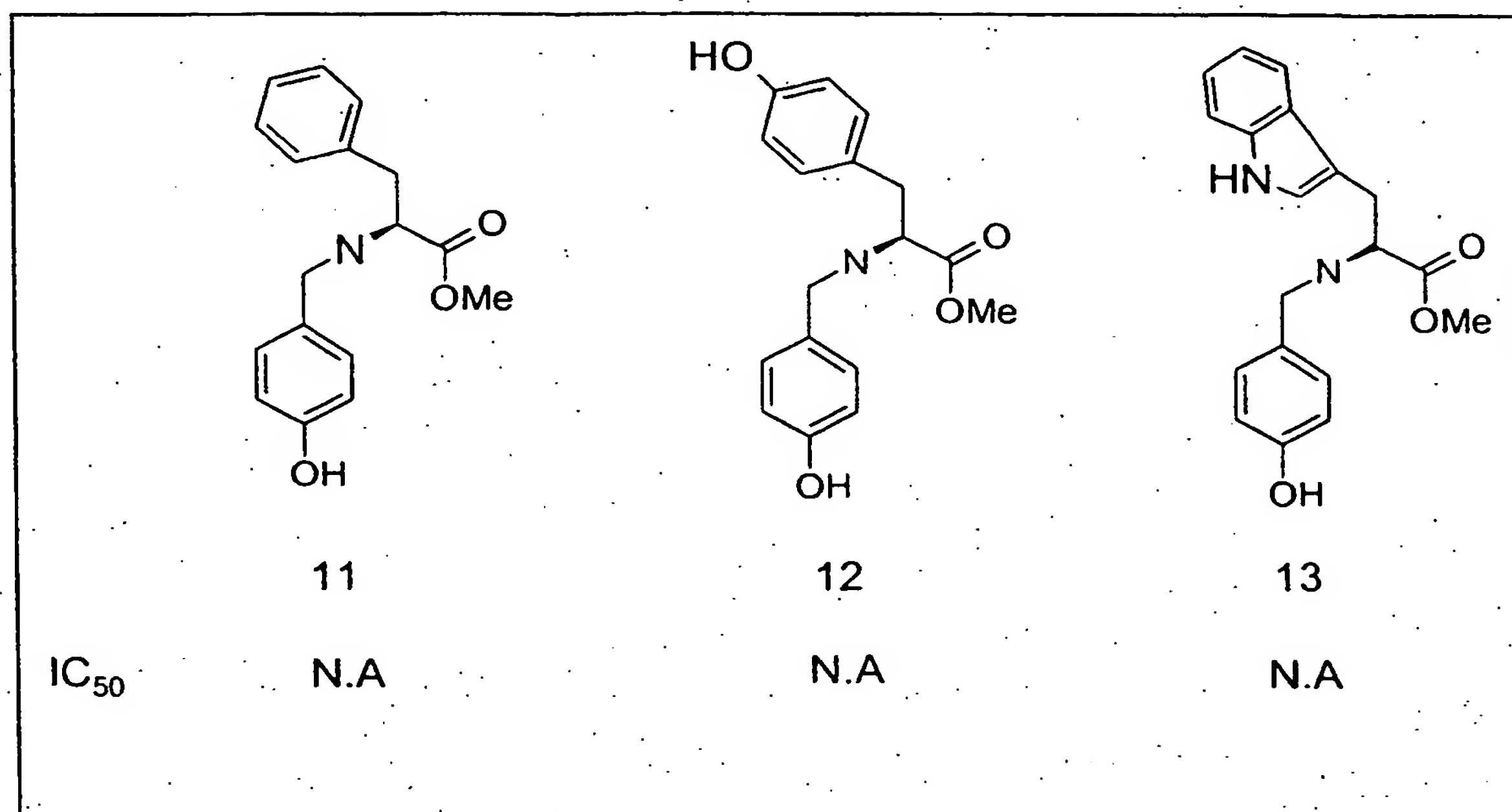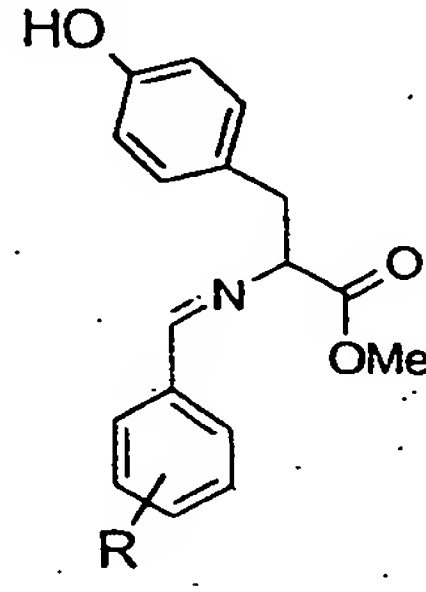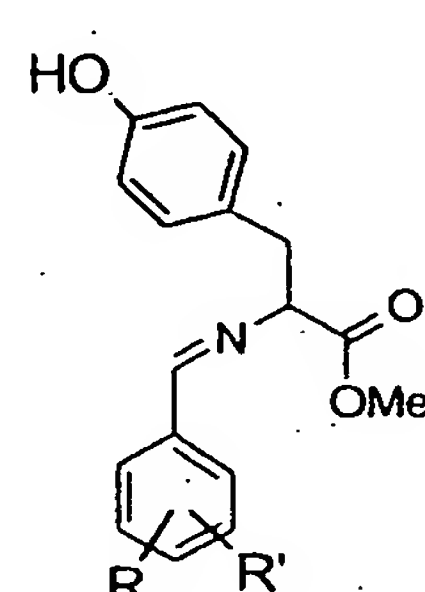
A further subset of compounds (compounds 14-16) is the condensation products of a protected aromatic amino acid with benzaldehyde.

14   N.A
15   104μM
16   N.A
IC$_{50}$
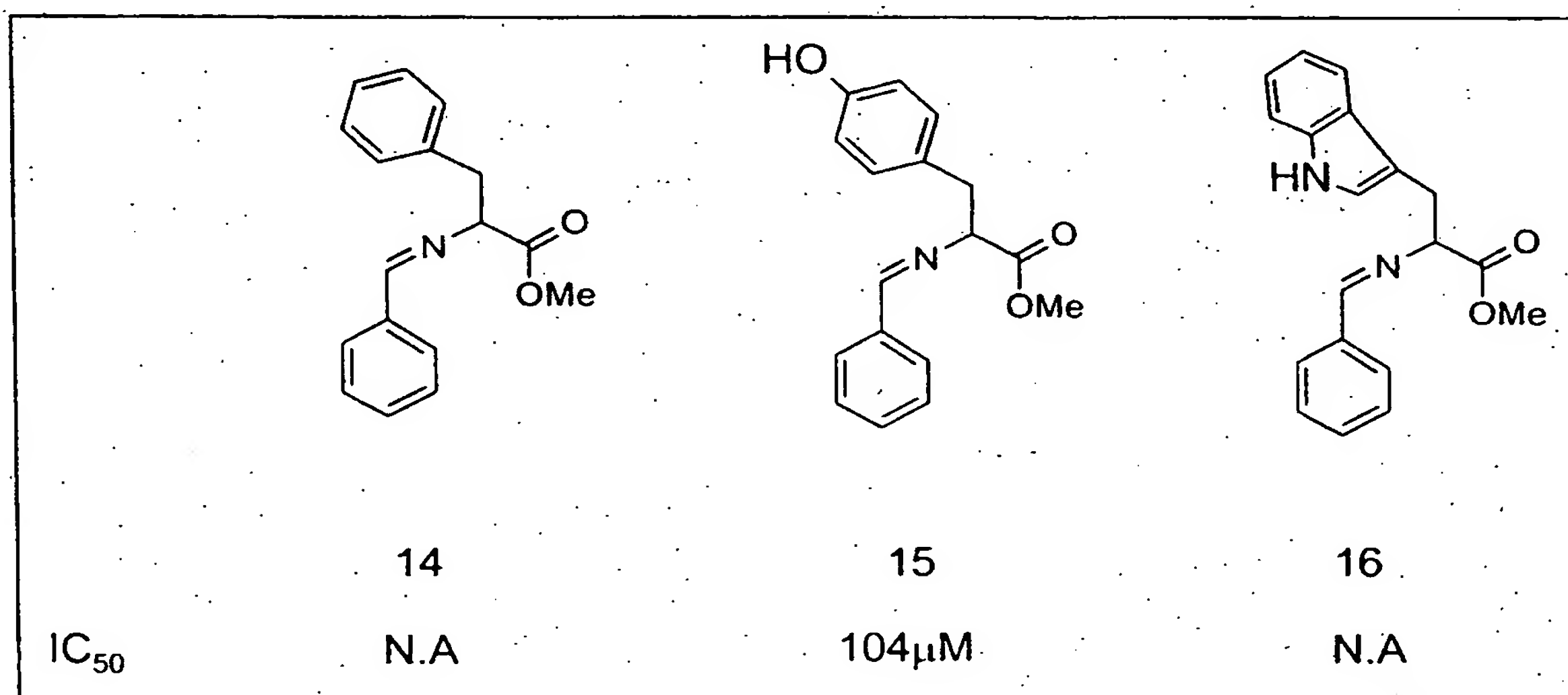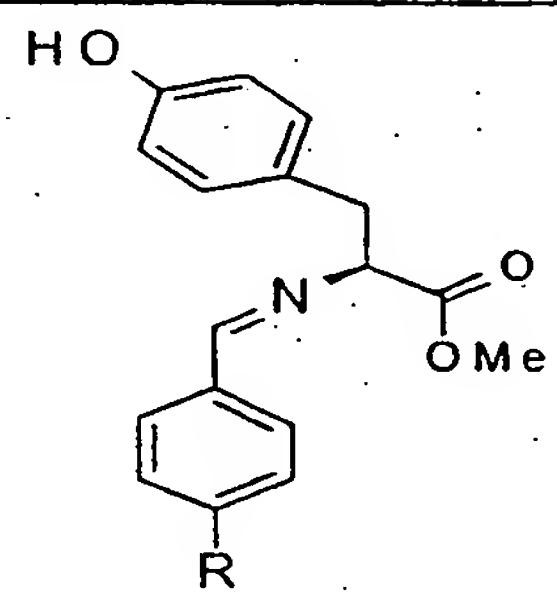
Additional substituted Schiff base compounds are illustrated below as compounds 17-21.

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R=3-OH	17	100μM
R=2-OH	18	>100μM
R=4-NO$_2$	19	N.A

| R=2-OH, R'=3-OH | 20 | >100μM |
| R=3-OH, R'=4-OH | 21 | >100μM |

15

20

Compounds 22-25 have a halogen substitution or a methyl substitution (instead of hydroxyl) at the para position of the benzaldehyde moiety, while the amino acid moiety corresponds to the methyl ester of L tyrosine.

25

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		Inhibition IC$_{50}$
R = Cl	22	N.A
R = Br	23	N.A
R = F	24	>100μM
R = CH$_3$	25	N.A

35

11

Compound 26, wherein Z is O-tertbutyl, X is N, R is the side chain of tyrosine, Y is O, R_2 is para-hydroxy, R_3 is H and R, X and Y together comprise a protected aromatic amino acid moiety, is shown below, together with the inhibitory value determined for this compound when present at a concentration of 100 μM in the MIF tautomerase assay.

HO

N

O

O

OH

26

% Inhibition= 70%
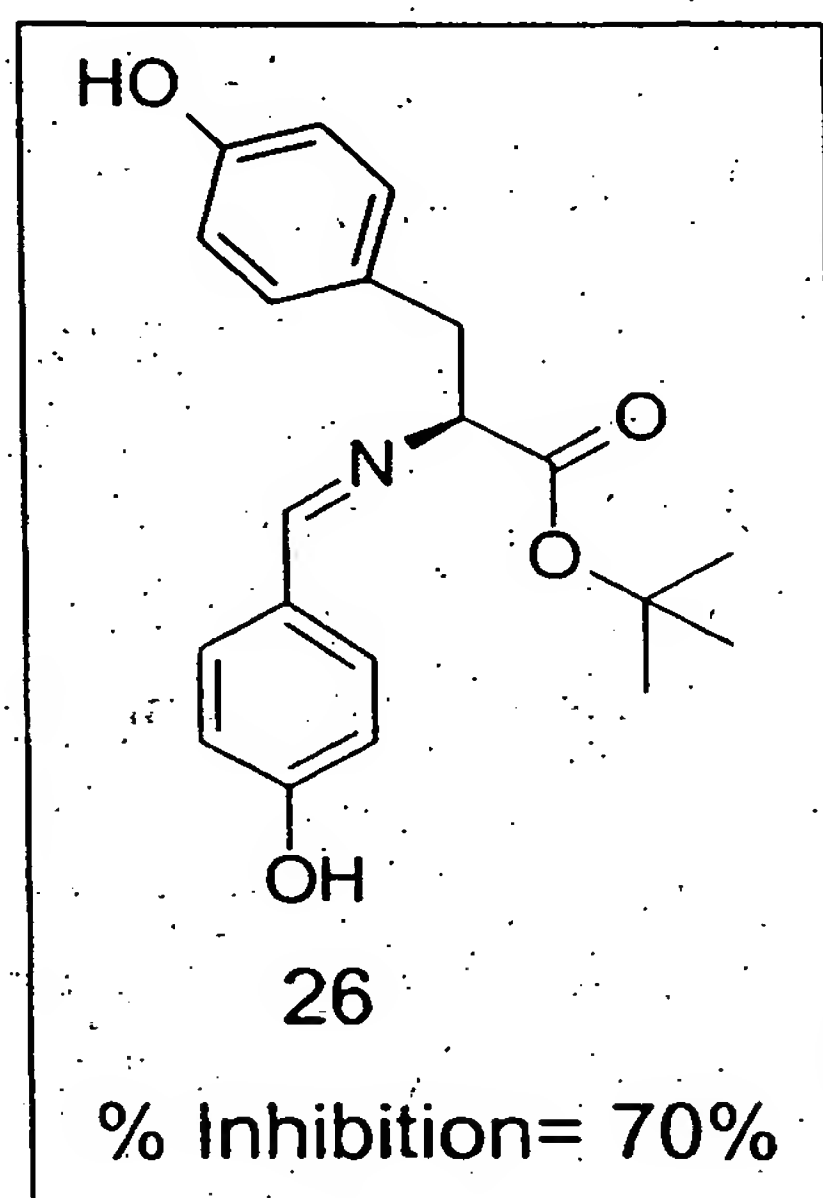
Compounds 27-29 (see below) comprise a protected L-Trp amino acid moiety together with a benzaldehyde moiety substituted with a halogen in the para position.

HN

N

O

OMe

R

		Inhibition IC_{50}
R=Cl	27	N.A
R=Br	28	N.A
R=F	29	>100μM

12
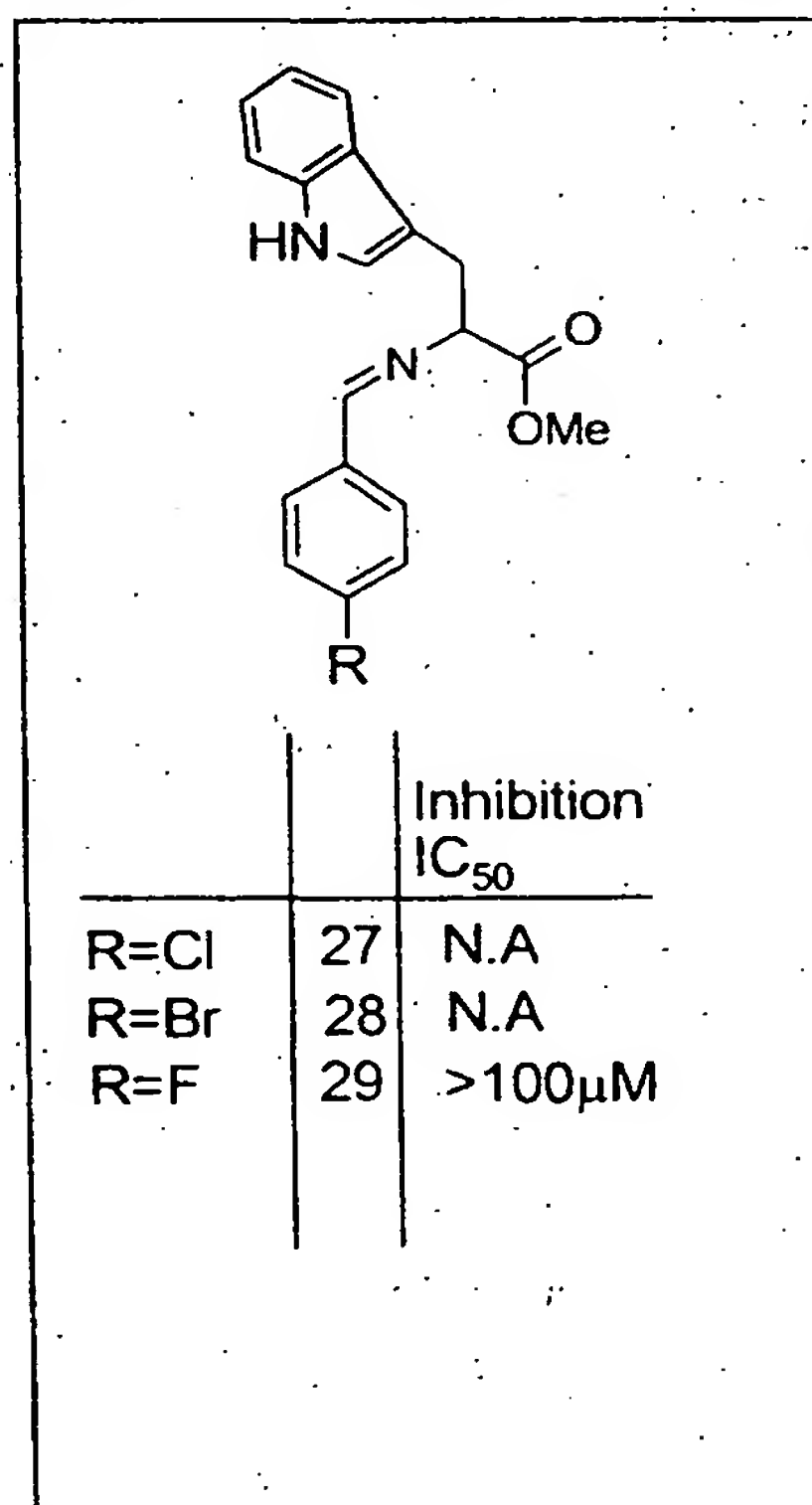
<u>Pharmaceutical Formulations</u>

The compounds of the present invention have utility in pharmacological compositions for the treatment and prevention of many diseases and disorders characterized by an MIF response, whereby MIF is released from cellular sources and MIF production is enhanced. A

5   compound of the invention can be administered to a human patient by itself or in pharmaceutical compositions where it is mixed with suitable carriers or excipients at doses to treat or ameliorate various conditions characterized by MIF release. A therapeutically effective dose further refers to that amount of the compound sufficient to inhibit MIF tautomerase activity and MIF bioactivity, it being understood that such inhibition may occur at

10   different concentrations such that a person skilled in the art could determine the required dosage of compound to inhibit the target MIF activity. Therapeutically effective doses may be administered alone or as adjunctive therapy in combination with other treatments for tumor growth or associated diseases. Techniques for the formulation and administration of the compounds of the instant application may be found in "*Remington's Pharmaceutical Sciences*"

15   Mack Publishing Co., Easton, PA, latest addition.

Suitable routes of administration may, for example, include topical, cutaneous, oral, rectal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections, and optionally in a depot or

20   sustained release formulation. In an embodiment, topical administration utilizes excipients such as creams, emulsifiers, and oils. Further embodiments, topical administration utilizes dermal absorption enhancers selected from the group consisting of dimethyl sulfoxide, menthol, lauryl alcohol, lauric acid, arachidonic acid and C_{10}-C_{20} polyhydroxy acids and thymol.

25   Furthermore, one may administer a compound of the present invention in a targeted drug delivery system, for example in a liposome.

The pharmaceutical compositions and compounds of the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, dragee-making, levitating, emulsifying, encapsulating, entrapping, or lyophilizing

30   processes. Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries that facilitate processing of the active compounds into preparations, which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

35   For injection, the compounds of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers, such as Hank's solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are known in the art.

For oral administration, the compounds can be formulated readily by combining the

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active compounds with pharmaceutically acceptable carriers well known to those in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained by combining the

5 compound with a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-

10 cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone,

15 carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations that can be used orally include push-fit capsules made of

20 gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In

25 addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present

30 invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g., gelatin for use

35 in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The

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compositions may take such forms as suspensions. solutions or emulsions in oily or aqueous vehicles. and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil. or synthetic fatty acid esters. such as ethyl oleate or triglycerides. or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension. such as sodium carboxymethyl cellulose, sorbitol. or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g.. sterile pyrogen-free water. before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas. e.g.. containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously. the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example. the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins. or as sparingly soluble derivatives. for example. as a sparingly soluble salt.

Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethylsulfoxide also may be employed. although usually at the cost of greater toxicity. Additionally, the compounds may be delivered using a sustained-release system. such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various forms of sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature. release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent. additional strategies for protein stabilization may be employed.

The pharmaceutical compositions also may comprise suitable solid- or gel- phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate. calcium phosphate. various sugars. starches. cellulose derivatives. gelatin. and polymers such as polyethylene glycols.

Many of the compounds of the invention identified as neutralizers of MIF activity may be provided as salts with pharmaceutically compatible counterions. Pharmaceutically compatible salts may be formed with many acids. including but not limited to hydrochloric.

sulfuric, acetic, lactic, tartaric. malic, succinic, etc.: or bases. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. Examples of pharmaceutically acceptable salts, carriers or excipients are well known to those skilled in the art and can be found, for example, in *Remington's Pharmaceutical Sciences*, 18th Edition, A.R.

5   Gennaro, Ed., Mack Publishing Co., Easton, PA, 1990. Such salts include, but are not limited to, sodium, potassium, lithium. calcium. magnesium, iron. zinc, hydrochloride, hydrobromide, hydroiodide, acetate, citrate, tartrate and malate salts, and the like.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve

10   their intended purpose. More specifically, a therapeutically effective amount means an amount effective to prevent or inhibit development or progression of a disease characterized by MIF release and production in the subject being treated. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

15   For any compound used in the method of the invention. the therapeutically effective dose can be estimated initially from tautomerase inhibition assays and cell culture assays. Such information can be used to more accurately determine useful doses in humans.

A therapeutically effective dose refers to that amount of the compound that results in a reduction in the development or severity of a disease characterized by MIF release and

20   production. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical, pharmacological, and toxicological procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the

25   ratio between LD_{50} and ED_{50}. Compounds that exhibit high therapeutic indices are preferred. The data obtained from cell culture assays or animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration

30   utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See *e.g.* Fingl et al., 1975, in *"The Pharmacological Basis of Therapeutics"*, Ch. 1 p. 1).

Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain the desired modulating effects, or minimal

35   effective concentration (MEC). The MEC will vary for each compound but can be estimated from *in vitro* data; *e.g.* the concentration necessary to achieve a 50-90% inhibition of MIF activity. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. However, HPLC assays, bioassays or immunoassays can be used to determine plasma concentrations.

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Dosage intervals can also be determined using the MEC value. Compounds should be administered using a regimen that maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%.

In cases of local administration, such as topical or cutaneous administration, or for

5   instance, direct introduction into a target organ or tissue, or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

The amount of composition administered will, of course, be dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration and the judgment of the prescribing physician.

10   The compositions may, if desired, be presented in a pack or dispenser device that may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be

15   prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

Synthesis

In the examples of the syntheses that follow, all reagents and solvents used were purchased at the highest commercial quality. All solvents used were HPLC grade from Fisher.

20   ^{1}H (270 MHz) and ^{13}CNMR (67.5 MHz) NMR spectra were recorded on a JEOL eclipse 270 spectrometer. Coupling constants were reported in Hertz (Hz), and chemical shifts were reported in parts per million (ppm) relative to tetramethylsilane (TMS, 0.0 ppm) with $CDCl_3$, DMSO or CD_3OD as solvent. Thin layer (TLC) and flash column chromatography were performed using Alumina B, F-254 TLC plates form Selecto Scientific and Fisher Scientific

25   Basic alumina Brockman activity I, respectively. The reactions were monitored by TLC and ^{1}HNMR and were stopped when the yield of the crude according to ^{1}HNMR was 90-95%.

Example 1

This example illustrates the synthesis of Schiff base compounds having aliphatic amino

30   acids. To a suspension of anhydrous $MgSO_4$ in CH_2Cl_2 was added the hydrochloride salt of the amino acid's methyl ester triethylamine (1.8 mol equiv) followed by 4-hydroxybenzaldehyde (1 mol equiv). The reactions were left stirring for 12 to 16 hours. The $MgSO_4$ was filtered off and the crude product was concentrated under reduced pressure. The crude product was re-dissolved in CH_2Cl_2, and washed twice with brine (2 x 20 ml). The

35   organic solvent was dried over $MgSO_4$ and decolorized with activated carbon. The crude was then purified by flash chromatography in basic alumina or silica treated with triethylamine to give the desired product.

For compound 1, glycine methyl ester hydrochloride (0.4 g, 3.2 mmol) was coupled to 4-hydroxybenzaldehyde (0.4 g, 3.2 mmol, 1 equiv) to give compound 1 (0.28 g, 40%).

¹HNMR(270 MHz, CD₃OD) δ 3.74 (3H, s), 4.36 (2H. s), 6.83 (2H, d, J=8.7 Hz), 7.63 (2H, d, J=8.7 Hz), 8.21 (1H, s); MS (ES) m/z 194 (M+H) base peak, 216 (M+Na).

For compound 2a, D-alanine methylester hydrochloride (0.2 g, 1.43mmol) was reacted with 4-hydroxybenzaldehyde (0.175g, 1.43mmol), which gave the compound as an off-white
5   solid (0.2 g, 67%).
¹HNMR(270 MHz, CD₃OD) δ 1.46 (3H, d, J=6.4 Hz), 3.72 (3H, s), 4.13 (1H, q, J=6.9 Hz), 6.82 (2H, d, J=8.7 Hz) 7.63 (2H, d, J=8.7 Hz), 8.23 (1H. s); MS (ES) m/z 208 (M+H) base peak, 230 (M+Na).

For compound 2b, L-alanine methylester hydrochloride (0.2 g, 1.43 mmol) was reacted
10   with 4-hydroxybenzaldehyde (0.175 g, 1.43 mmol), which gave compound 2b (0.2 g, 67%).
¹HNMR(270 MHz, CD₃OD) δ 1.46 (3H, d, J=6.4 Hz), 3.72 (3H, s), 4.13 (1H, q, J=6.9 Hz), 6.82 (2H, d, J=8.7 Hz) 7.63 (2H, d, J=8.7 Hz), 8.23 (1H. s); MS (ES) m/z 208 (M+H) base peak, 230 (M+Na).

For compound 3, L-valine methylester hydrochloride (0.4 g, 2.5 mmol) was coupled to
15   4-hydroxybenzaldehyde (0.3 g, 2.5 mmol) to give compound 3 (0.2 g, 36%).
¹HNMR(270 MHz, CD₃OD) δ 0.87 (3H, d, J=8.1 Hz), 0.94 (3H, d, J= 6.9 Hz), 2.23-2.36 (1H, m), 3.63 (1H, d, J=7.7 Hz), 3.73 (3H, s), 6.82 (2H, d, J=8.6 Hz), 7. 65 (2H, d, J=8.6 Hz), 8.18 (1H, s); MS (ES) m/z 236 (M+H) base peak, 258 (M+Na).

Compound 4 (0.2 g, 48%), was obtained from L-leucine methyl ester hydrochloride
20   (0.3 g, 1.7 mmol) and 4-hydroxybenzaldehyde (0.2 g, 1.7 mmol).
¹HNMR(270 MHz, CD₃OD) δ 0.91 (3H, d, J=6.4), 0.94 (3H, d, J=6.7 Hz), 1.72-1.81 (2H, m), 3.65-3.70 (1H, m), 3.72 (3H, s), 4.08 (1H, dd, J= 7.2, 7.3 Hz), 6.83 (2H, d, J=8.6 Hz), 7.64 (2H, d, J=8.6), 8.23 (1H, s); MS (ES) m/z 250 (M+H) base peak, 272 (M+Na).

For compound 5, L-isoleucine methyl ester hydrochloride (0.3 g, 1.7 mmol) was
25   combined with 4-hydroxybenzaldehyde (0.2 g, 1.7mmol) to give 5 (0.23 g, 55%).
¹HNMR(270 MHz, CD₃OD) δ 0.85-0.94 (6H, m), 1.17-1.24 (1H, m), 1.44-1.51 (1H, m), 2.01-2.14 (1H, m), 3.72 (3H, s), 3. 73 (1H, d), 6.83 (2H, d, J=8.6 Hz), 7.64 (2H, d, J=8.6), 8.23 (1H, s); MS (ES) m/z 250 (M+H) base peak, 272 (M+Na).

For compound 6, L-methionine methyl ester hydrochloride (0.4 g, 2.0 mmol) and 4-
30   hydroxybenzaldehyde (0.25 g, 2.0 mmol) were combined to give compound 6 (0.23 g, 43%).
¹HNMR(270 MHz, CD₃OD) δ 2.04 (3H, s), 2.04-2.60 (4H, m), 3.73 (3H, s), 4.18 (1H, dd, J=4.9, 8.9Hz), 6.83 (2H, d, J=8.6 Hz), 7.64 (2H, d, J=8.6), 8.24 (1H, s); MS (ES) m/z 268 (M+H) base peak, 290 (M+Na).

For compound 7, L-serine methyl ester hydrochloride (0.4 g, 2.6 mmol) and 4-
35   hydroxybenzaldehyde (0.35 g, 2.6 mmol) were combined to give compound 7 (0.1 g, 20%).
¹HNMR(270 MHz, CD₃OD) δ 3.74 (3H, s), 3.79 (1H, dd, J= 11.0, 7.1 Hz), 4.00 (1H, dd, J= 4.9, 11.0 Hz), 4.10 (1H, dd, J=7.2, 5.0 Hz), 6.81 (1H, d, J=8.9 Hz), 7.65 (1H, d, J=8.6), 8.23 (1H, s); MS (ES) m/z 224 (M+H), 246 (M+Na) base peak.

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Example 2

This example provides syntheses of compounds comprising Schiff base condensation products of aromatic amino acids with a benzyaldehyde reactant. To a suspension of anhydrous $MgSO_4$ (0.2 g) and the hydrocloride salt of L-phenylalanine methyl ester (0.3 g, 1.4 mmol) in CH_2Cl_2 (4.8 mL), triethylamine (0.35 ml, 2.5 mmol) was added followed by 4-hydroxybenzaldehyde (0.17 g, 1.4 mmol). The reaction was left stirring for 24 hrs. The $MgSO_4$ was filtered off and the crude product was concentrated under reduced pressure. The crude product was re-dissolved in a mixture of CH_2Cl_2/EtOAc, (40/60), and washed twice with brine (2x20 mL). The organic phase was dried over $MgSO_4$ and decolorized with activated carbon. The crude product was then purified by flash chromatography in basic alumina to give the desired product, compound 8 (0.23 g, 58%).

^{1}HNMR(270 MHz, CD₃OD) δ 3.03 (1H, dd, J=9.1, 13.3 Hz), 3.32 (1H, dd, J=5.2, 13.3 Hz), 3.72 (3H, s), 4.17 (1H, dd, 5.2, 8.9 Hz), 6.78 (2H, d, J=8.6 Hz), 7.16-7.21 (5H, m), 7.52 (2H, d, J=8.7 Hz), 7.8 (1H, s); ^{13}CNMR(67.5 MHz, CD₃OD) δ 39.3, 51.3, 73.0, 115.2, 126.3, 128.0, 129.4, 130.3, 165.1, 172.4; MS (ES) m/z 284 (M+H) base peak, 306 (M+Na).

Compound 9 was prepared as above except that a suspension of L-tyrosine methyl ester (0.4 g, 2.1 mmol) was added to a suspension of anhydrous $MgSO_4$ (0.25 g) in CH_2Cl_2 (4.8 ml) followed by 4-hydroxybenzaldehyde (0.25 g, 2.1 mmol). The reaction was left stirring for 48 hrs. The $MgSO_4$ was filtered off and the crude product was concentrated under reduced pressure to give the desired product compound 9 (0.27 g, 61%).

^{1}HNMR(270 MHz, CD₃OD) δ 2.92 (1H, dd, J= 8.9, 13 Hz), 3.25 (1H, dd), 3.71 (3H, s), 4.10 (1H, dd, J=5.2, 8.9), 6.63 (2H, d, J=8.4 Hz), 6.80 (2H, d, J=8.6 Hz), 6.96 (2H, d, J=8.6 Hz), 7.52 (2H, d, J=8.7 Hz), 7.82 (1H, s); ^{13}CNMR(67.5 MHz, CD₃OD) δ 38.6, 51.2, 74.9, 114.7, 115.2, 126.7, 127.9, 130.3, 130.5, 155.8, 160.8, 165.0, 172.7; MS (ES) m/z 299 (M+H), 322 (M+Na), base peak.

For compound 10, the hydrocloride salt of L-tryptophan methyl ester (0.3 g, 1.2 mmol) was added to a suspension of anhydrous $MgSO_4$ (0.2 g) in CH_2Cl_2 (4.6 m). Triethylamine (0.3 ml, 2.1 mmol) was added, followed by 4-hydroxybenzaldehyde (0.15 g, 1.2 mmol). The reaction was left stirring for 48 hrs. The $MgSO_4$ was filtered off and the crude product was concentrated under reduced pressure. The crude product was re-dissolved in a mixture of CH_2Cl_2/EtOAc, (40/60), and washed twice with brine (2x20 ml). The organic was dried over $MgSO_4$ and decolorized with activated carbon. The crude product was then purified by flash chromatography in basic alumina to give the desired product compound 10 (0.20 g, 53%).

^{1}HNMR(270 MHz, CD₃OD) δ 3.12 (1H, dd, J= 8.9, 14.4 Hz), 3.50 (1H, dd, J= 5.2, 14.4 Hz), 3.73 (3H, s), 4.22 (1H, dd, J= 5.2, 8.7 Hz), 6.70-7.54 (9H, m), 7.76 (1H, s); ^{13}CNMR(67.5 MHz, CD₃OD) δ 29.3, 51.2, 73.6, 109.8, 110.9, 115.1, 118.0, 118.4, 121.0, 123.6, 127.2, 130.3, 136.7, 164.6, 173.1;MS (ES) m/z 323 (M+H) base peak, 3345 (M+Na).

For compound 8b, triethylamine (0.35 ml, 2.5 mmol) was added followed by 4-hydroxybenzaldehyde (0.17 g, 1.4 mmol) to a suspension of anhydrous $MgSO_4$ (0.2 g) and the

hydrocloride salt of D-phenylalanine methyl ester (0.3 g, 1.4 mmol) in CH_2Cl_2 (4.8 ml). The reaction was left stirring for 24 hrs. The $MgSO_4$ was filtered off and the crude was concentrated under reduced pressure. The crude was then purified by flash chromatography in basic alumina to give the desired product compound **8b** (0.32 g, 82%%).

^{1}HNMR(270 MHz, CD_3OD) δ 3.03 (1H, dd, J=9.1, 13.3 Hz), 3.32 (1H, dd, J=5.2, 13.3 Hz), 3.72 (3H, s), 4.17 (1H, dd, 5.2, 8.9 Hz), 6.78 (2H, d, J=8.6 Hz), 7.16-7.21 (5H, m), 7.52 (2H, d, J=8.7 Hz), 7.8 (1H, s);

For compound **10b**, triethylamine (0.3 mL, 2.1 mmol) was added followed by 4-hydroxybenzaldehyde (0.15 g, 1.2 mmol) to a suspension of anhydrous $MgSO_4$ (0.2 g) and the hydrocloride salt of D-tryptophan methyl ester (0.3 g, 1.2 mmol) in CH_2Cl_2 (4.6 ml). The reaction was left stirring for 48 hrs. The $MgSO_4$ was filtrated off and the crude was then purified by flash chromatography in basic alumina to give the desired product compound **10b** (0.28 g, 74%).

^{1}HNMR(270 MHz, CD_3OD) δ 3.12 (1H, dd, J= 8.9, 14.4 Hz), 3.50 (1H, dd, J= 5.2, 14.4 Hz), 3.73 (3H, s), 4.22 (1H, dd, J= 5.2, 8.7 Hz), 6.70-7.54 (9H, m), 7.76 (1H, s).

Example 3

This example provides syntheses of compounds wherein the Schiff base condensation products of protected amino acids with parahydroxybenzaldehyde are reduced. A general synthesis procedure follows the previous procedure except that the purified imine product was suspended in absolute ethanol and $NaBH_4$ (1 mol equiv) was added to each reaction mixture. After 1hr, the reactions were quenched with water and extracted with CH_2Cl_2 to give the reduced compounds in quantitative yields once the organic fractions were dried over $MgSO_4$ and evaporated to dryness. Further purification through silica flash chromatography was done.

For compound **11**:

^{1}HNMR(270 MHz, CD_3OD) δ 2.92 (2H, m), 3.45-3.66 (6H, m), 6.69 (2H, d, J= 8.4), 6.98-7.30 (7H, m); ^{13}CNMR(67.5 MHz, CD_3OD) δ 38.8, 50.8, 50.9, 61.7, 114.8, 126.5, 128.1, 128.9, 129.5, 129.6, 137.1, 156.5, 174.6.

For compound **12**:

^{1}HNMR(270 MHz, CD_3OD) δ 2.87 (2H, m), 3.54-3.62 (6H, m), 6.65-6.74 (4H, m), 6.93 (2H, d, J=8.6 Hz), 7.08 (2H, d, J=8.6 Hz); ^{13}CNMR(67.5 MHz, CD_3OD) d 37.4, 50.8, 51.1, 61.6, 115.0, 126.8, 127.8, 129.9, 156.2, 156.9, 173.5

For compound **13**:

^{1}HNMR(270 MHz, CD_3OD) δ 3.11 (2H, m), 3.45-3.65 (6H, m), 6.50-7.42 (7H, m); ^{13}CNMR(67.5 MHz, CD_3OD) δ 28.6, 50.9, 51.0, 60.8, 109.4, 111.0, 114.8, 117.9, 118.4, 121.1, 123.1, 127.3, 129.5, 136.7, 156.4, 174.9.

Example 4

This example provides syntheses of compounds comprising condensation products of

protected amino acids with benzaldehyde. For compound 14. the same general procedure was followed as for compound 8 to give compound 14 (0.2 g, 61%).

^{1}HNMR(270 MHz, CD$_3$OD).δ 3.05 (1H, dd, J=9.1, 13.6 Hz). 3.24 (1H, dd, J= 4.8, 13.6 Hz). 3.73 (3H, s), 4.24 (1H, dd, J=4.9, 9.1 Hz); 7.13-7.19 (4H, m), 7.30-7.50 (3H, m), 7.66 (2H, m), 7.96 (1H, s).

For compound 15, the same general procedure was followed as for compound 9 to obtain 15 (0.4 g, 90%).

^{1}HNMR(270 MHz, CD$_3$OD) δ 2.94 (1H, dd, J=8.9, 13.9 Hz), 3.24 (1H, dd, J=4.9, 13.9), 3.73 (3H, s), 4.16 (1H, dd, J=4.9, 8.9), 6.61 (2H, d, J= 8.6 Hz), 6.96 (2H, d, J=8.4), 7.38-7.50 (3H, m) 7.64-7.70 (2H, m), 7.94 (1H, s).

For compound 16, the same general procedure was followed as for compound 10 to give compound 16 (0.16 g, 46%).

^{1}HNMR(270 MHz, CD$_3$OD) δ 3.15 (1H, dd, J=8.9, 14 Hz), 3.49 (1H, dd, J=4.7, 14 Hz), 3.73 (3H, s), 4.28 (1H, dd, J=4.7, 8.4 Hz), 6.92-7.63 (10H, m), 7.82 (1H, s).

Example 5

This example provides syntheses of compounds that feature differently substituted benzaldehyde moieties. A general synthesis provides that a benzaldehyde with the appropriate substituent/s was added (1 mol equiv) to a suspension in CH$_2$Cl$_2$ of anhydrous MgSO$_4$ and L-tyrosine methyl ester. The reaction was left stirring for 48 hrs. The MgSO$_4$ was filtered off and the crude product was concentrated under reduced pressure to give the desired product.

Compound 17 was obtained (0.42 g, 91%).

^{1}HNMR(270 MHz, CD$_3$OD) δ 2.93 (1H, dd, J=8.9, 13.6 Hz), 3.23 (1H, dd, J=4.9, 13.4 Hz), 3.72 (3H, s), 4.13 (1H, dd, J=4.9, 8.9 Hz), 6.61-7.28 (8H, m) 7.85 (1H, s).

Compound 18 was obtained pure (0.38 g, 82%).

^{1}HNMR(270 MHz, CD$_3$OD) δ 2.97 (1H, dd, J=8.9, 13.6 Hz), 3.23 (1H, dd, J=4.9, 13.4 Hz), 3.73 (3H, s), 4.21 (1H, dd, J=4.9, 8.7 Hz), 6.62-7.34 (8H, m) 8.06 (1H, s).

Compound 19 was obtained pure (0.38 g, 75%).

^{1}HNMR(270 MHz, CD$_3$OD) δ 2.97 (1H, dd, J=8.9, 13.3 Hz), 3.26 (1H, dd, J=4.7, 13.9 Hz), 3.73 (3H, s), 4.25 (1H, dd, J=4.7, 8.9 Hz), 6.62 (2H, d, J=8.4 Hz), 6.94 (2H, d, J=8.6 Hz), 7.91 (2H, d, J=8.9 Hz), 8.03 (1H, s), 8.26 (2H, d, J=8.9 Hz).

Compound 20 was obtained (0.47 g, 98%).

^{1}HNMR(270 MHz, CD$_3$OD) δ 2.28 (1H, dd, J=8.6, 13.6 Hz), 3.24 (1H, dd, J=8.9, 13.6 Hz), 3.73 (3H, s), 4.23 (1H, dd), 6.61- 69.8 (7H, m), 8.02 (1H, s).

Compound 21 was obtained (0.41 g, 85%).

^{1}HNMR(270 MHz, CD$_3$OD) δ 2.91 (1H, dd, J=8.9, 13.6 Hz), 3.20 (1H, dd, J=5.2, 13.8 Hz), 3.71 (3H, s), 4.09 (1H, dd, J=4.5, 9.3 Hz), 6.61-6.98 (6H, m), 7.18 (1H, s), 7.74 (1H, s).

Example 6

This example provides syntheses of compounds that feature differently substituted benzaldehyde moieties. A general synthesis provides that a benzaldehyde with the appropriate substituent was added (1 mol equiv) to a suspension in CH_2Cl_2 of anhydrous $MgSO_4$ and L-tyrosine methyl ester. The reaction was left stirring for 48 hrs. The $MgSO_4$ was filtered off and the crude product was concentrated under reduced pressure to give the desired product.

After condensation, compound 22 was obtained (0.38 g, 78%).

^{1}HNMR(270 MHz, CD_3OD) δ 2.92 (1H, dd, J=8.9, 13.4 Hz), 3.20 (1H, dd, J=4.9, 13.6 Hz), 3.70 (3H, s), 4.14 (1H, dd, J=4.9, 8.9 Hz), 6.64 (2H, d, J=8.4 Hz), 6.94 (2H, d, J=8.4), 7.37 (2H, d, J=8.6 Hz), 7.62 (2H, d, J=8.4), 7.87 (1H, s).

After condensation, compound 23 was obtained (0.29 g, 52%).

^{1}HNMR(270 MHz, CD_3OD) δ 2.95 (1H, dd, J=4.5, 13.6 Hz), 3.22 (1H, dd, J=5.2, 13.6 Hz), 3.70 (3H, s), 4.15 (1H, dd, J=4.9, 8.9 Hz), 6.63 (2H, d, J=8.4), 6.94 (2H, d, J=8.6), 7.55 (4H, s), 7.86 (1H, s).

Compound 24 was obtained following the general procedure (0.12 g, 25%).

^{1}HNMR(270 MHz, CD_3OD) δ 2.92 (1H, dd, J=9.1, 13.6 Hz), 3.22 (1H, dd, J=5.2, 13.6 Hz), 3.70 (3H, s), 4.16 (1H, dd, J=5.2, 9.2 Hz), 6.63 (2H, d, J=8.6 Hz), 6.95 (2H, d, J=8.7 Hz), 7.11-7.18 (2H, m), 7.70-7.73 (2H, m), 7.92 (1H, s). ^{13}CNMR (67.5 MHz, CD_3OD) δ 38.6, 50.0, 74.8, 114.7, 115.2, 115.5, 127.7, 130.5, 135.6, 155.9, 163.6, 172.5.

Compound 25 was obtained following the general procedure (0.23 g, 51%).

^{1}HNMR(270 MHz, CD_3OD) δ 2.35 (3H, s), 2.94 (1H, dd, J=9.2, 13.6 Hz), 3.24 (1H, dd, J=5.2, 13.6 Hz), 3.71 (3H, s), 4.13 (1H, dd, J=5.2, 8.9 Hz), 6.63 (2H, dd, J=8.4 Hz), 6.95 (2H, dd, J=8.7 Hz), 7.21 (2H, d, J=7.9 Hz), 7.56 (2H, d, J=8.2 Hz), 7.88 (1H, s).

Compound 26 exemplifies a further type of compound in which the protecting group of the amino acid moiety is tert butyl. 4-hydroxybenzaldehyde (0.26 g, 2.1 mmol) was added to a suspension in CH_2Cl_2 (7 ml) of anhydrous $MgSO_4$ (0.35 g) and L-tyrosine t-butyl ester (0.5 g, 2.1 mmol). The reaction was left stirring for 48 hrs. The $MgSO_4$ was filtered off and the crude was concentrated under reduced pressure to give the desired product compound 26 (0.29 g, 49%).

^{1}HNMR(270 MHz, CD_3OD) δ 1.39 (9H, s), 3.14 (1H, dd, J=8.6, 13.4 Hz), 3.16 (1H, dd, J=5.9, 4.7 Hz), 3.34 (3H, s) 3.99 (1H, dd), 6.64 (2H, d, J=8.7), 6.80 (2H, d, J=8.7 Hz), 6.98 (2H, d, J=8.6 Hz), 7.55 (2H, d, J=8.6), 7.87 (1H, s).

Example 7

This example provides syntheses of further compounds that comprise a halo-substituted benzaldehyde moiety. A general synthesis provides that triethylamine (1.8 mol equiv) and the desired halo-substituted parabenzaldehyde (1 mol equiv) were added to a preparation of the hydrochloride salt of L-tryptophan methyl ester in a suspension of anhydrous $MgSO_4$ in

CH$_2$Cl$_2$. The reactions were left stirring for 24hrs. The MgSO$_4$ was filtered off and the crude product was concentrated under reduced pressure. The crude product was then purified by flash chromatography in basic alumina to give the desired product.

Compound **27** was obtained following the general procedure (0.17 g, 42%).

^{1}HNMR(270 MHz, CD$_3$OD) δ 3.13 (1H, dd, J=8.9, 14.3 Hz), 3.45 (1H, dd, J=4.7, 14.3 Hz), 3.67 (3H, s), 4.21 (1H, dd, J=4.7, 8.9 Hz) 6.80-7.54 (9H, m), 7.65 (1H,s).

Compound **28** was obtained following the general procedure (0.22 g, 48%).

^{1}HNMR(270 MHz, CD$_3$OD) δ 3.16 (1H, dd, J=8.9, 14.6 Hz) 3.48 (1H, dd, J=5.2, 14.6 Hz), 3.74 (3H, s), 4.29 (1H, dd, J=5.2, 8.9 Hz), 6.94-7.08 (4H, m), 7.28 (1H, d, J= 7.7 Hz), 7.54 (4H, s), 7.81 (1H, s).

After condensation, compound **29** was obtained (0.22 g, 58%).

^{1}HNMR(270 MHz, CD$_3$OD) δ 3.15 (1H, dd, J=8.9, 14.3 Hz), 3.51 (1H, dd, J=4.9, 14.3 Hz), 3.7 (1H, dd), 4.24 (1H, dd, J=4.9, 8.9 Hz), 6.80-7.64 (9H, m), 7.75 (1H, s).

Example 8

Schiff base conjugation products, especially conjugated imines, are of moderate stability. Although lability has not been problematic with the amino acid/benzaldehyde Schiff base (AA/BSB) compounds of the present invention. Schiff base condensation products can hydrolyze to yield the original free amine and aldehyde reactants. Compounds of the present invention, having an imine bond between the amino acid and the benzaldehyde moieties, showed greater potency as inhibitors of MIF activity than did corresponding compounds wherein the imine bond was specifically reduced, suggesting that unsaturation at this linkage is a relevant feature. Since carba analogs of the Schiff base condensation products can be expected to exhibit increased stability relative to their imine counterparts, carba analogs of the AA/BSB's can be prepared and evaluated. For example, the imine bond (carbon-nitrogen double bond) of the above exemplified compounds can be substituted by a carbon-carbon double bond or by a carbon-carbon triple bond providing, respectively, 2-substituted-4-phenyl-but-3-enoic and 2-substituted-4-phenyl-but-3-ynoic acids and methyl esters thereof as carba analogs of amino acid/benzaldehyde Schiff base condensation products described in detail above.

As a general approach, L- and D- amino acids are utilized as a starting point. L- D-aromatic amino acids, for instance, are transformed to R and S-2-bromo-propionic acid, respectively, as described previously (Olah et al., *Chimica Acta* 66:1028-1030, 1983; Cushman et al., *Biochemistry* 16:5484-5491, 1977). The aromatic L-amino acids phenylalanine, tyrosine and tryptophan, for instance, are individually dissolved in 47% hydrobromic acid in water and then treated with sodium nitrite at 0 °C. The reaction stirs for two hours at the same temperature and the product is extracted in ethyl ether. This method delivers the bromo derivative of each individual amino acid in moderate to high yield (45-88%). Next, the nucleophilic substitution of the bromo by the phenylacetylenic carbanion lithium salt produces

the first desired compounds, namely the acetylenic derivatives (Inhardt and Fried, *Synthesis* 4, 285-291, 1990). Phenylacetylene is commercially available and the other substituted phenylacetylenes are prepared via Heck reaction. For instance, 4-Iodophenol is subjected to palladium-catalyzed substitution with ethynyltrimethylsilane followed by the removal of

5   trimethylsilyl group using refluxing methanolic KOH to form 4-hydroxyphenylacetylene (Sonogashira et al., *Tetrahedron Letters* 16:4467-4470, 1975; Tischler and Lanza, *Tetrahedron Letters* 27:1653-1656, 1986).

These acetylenic carba analogs are conveniently reduced to provide the desired olefinic carba analogs. Acetylenic bond reduction is well established and is of particular interest

10   because specific reduction conditions can be employed to selectively generate the Z- or the E-geometric isomers. Using Lindlar catalyst (Pd-CaCO$_3$-PbO) in ethanol and one atmosphere of hydrogen, for instance, yields the Z-alkene with little or no Z-E isomerization (Denmark and Jones, *Organic Chemistry* 47:4595-4597, 1982). The E-isomer is obtained by metal reduction, for instance Na in NH$_3$ or lithium aluminum hydride. Moreover, it has been found that Red-Al

15   (NaAlH$_2$(OCH$_2$CH$_2$OMe)$_2$) is the fastest, highest yielding and more stereo-selective reduction among the metal hydrides examined (Lambert et al., *Eur. J. Org. Chem.* 4:775-779, 1999; Schreiber et al., *J. Amer. Chem. Soc.* 109:1525-1529, 1987), such that this is a suitable reducing reagent to arrive at the E-isomer.

It is particularly noteworthy that specific interaction of the acetylenic derivatives with

20   MIF results in allene formation. Most likely, this reflects carbanion generation via abstraction of the alpha-proton by a basicity within the MIF active site, followed by rearrangement to a conjugated allene. Consequently, after some turnover, the active site proline-1 residue of MIF captures the allene molecule alkylatively, leading to an enzymatically inactive protein (Jung et al., *Biochemistry* 17:2628-2632, 1978) with a corresponding loss of MIF biological activity.

25   Alternatively, synthesis of 2-benzyl-4(4-hydroxyphenyl)-but-3-enoic acid methyl ester (the carba analog of phenylalanine/para-hydroxybenzaldehyde Schiff base, compound 30) is accomplished according to a general approach involving Heck's reaction, as illustrated by Scheme 1:

Scheme 1: Synthesis of the carba analog of phenylalanine/p-hydroxybenzaldehyde Schiff base (compound 30)

compound 30
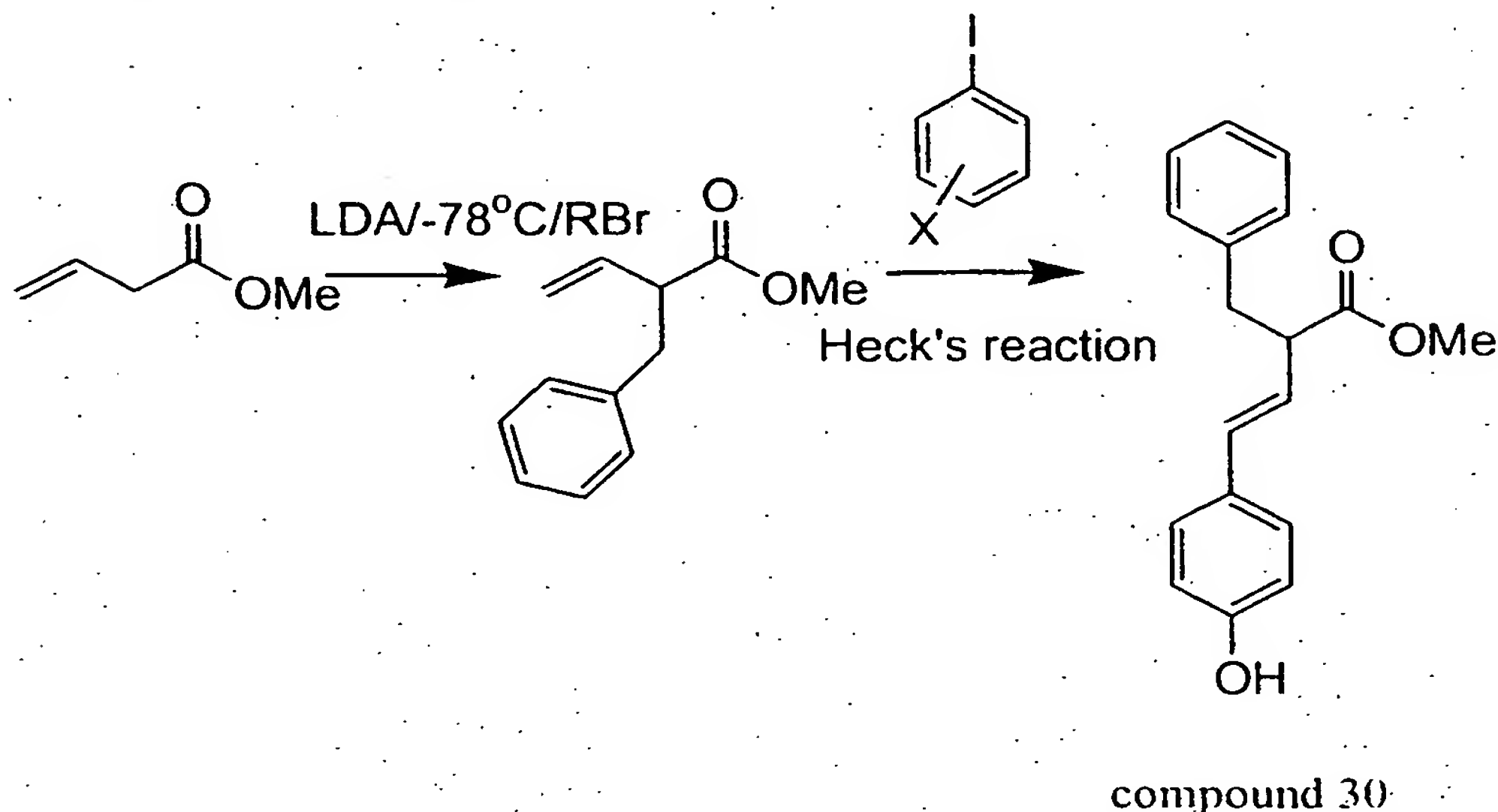
In this specific example, methyl-3-butenoate (0.53 mL, 5 mmol) is syringed into a THF
5   (20 mL) solution of lithium diisopropylamine (LDA; 5 mmol) at -78°C. After 30 minutes,
benzyl bromide (0.4 mL, 3.3 mmol) dissolved in THF (1.5 mL) is added dropwise and the
reaction mixture is warmed up to -30°C. The reaction is allowed to proceed for 2 hrs and then
quenched by adding aqueous ammonium chloride. After the reaction mixture warms to room
temperature, it is extracted with ether (3 x 20 mL) and the organic phase is dried over
10   magnesium sulfate. After flash chromatography, the olefinic compound is obtained in 82%
yield (0.52 g). Coupling with p-iodophenol under Heck's reaction conditions furnishes
quantitatively the desired compound 30. This synthetic approach is easily generalized to
provide additional carba analogs of amino acid/(optionally substituted)benzaldehyde Schiff
base antagonists of MIF of the present invention according to Formula I by: (i) varying the
15   RBr reagent (above) to provide side chain functionalities corresponding to the amino acid
component of the MIF antagonists of Formula I; and/or (ii) varying the iodophenol reagent
(above) to provide variously derived benzaldehyde components of the MIF antagonists of
Formula I.

20

Example 9

This example provides comparative pharmacologic activity data of the foregoing
compounds. As described in detail above (see particularly in the Detailed Description) an
assay for MIF tautomerase activity that is predictive of biological MIF inhibitory activity *in*

vivo was used to evaluate the test compounds and identify those predicted to have MIF inhibitory activity. Briefly, the assay procedure involves measuring MIF tautomerase activity in the presence of test compound (potential inhibitors) using an L-dopachrome methyl ester as substrate. Various concentrations of inhibitor compounds were added and an IC_{50} determined

5   for enzyme (tautomerase) inhibitory activity. These IC_{50} values, together with other indices of inhibitory activity (for example, percent inhibition at 100 μM of test compound) are reported in connection with the structural diagrams of the test compounds in the Detailed Description. Compounds are additionally assessed for inhibition of MIF biological activities in any of a number of assays for MIF activity including, for example, inhibition of MIF binding to target

10 cells, inhibition of MIF release or synthesis, inhibition of MIF immunoreactivity with MIF-specific antibodies, alterations of MIF conformation or structural integrity as assessed by circular dichroism spectroscopy or thermal stability measurement, inhibition of the pro-proliferative effects of MIF on quiescent NIH/3T3 cells and inhibition of the associated prolonged ERK activation therein, inhibition of MIF-induced arachadonic acid release from

15 NIH/3T3 cells, inhibition of MIF-induced fructose 2,6 bisphosphate formation in L6 myocytes, inhibition of MIF toxicity in TNF or LPS-challenged test animals, inhibition of the glucocorticoid counter-regulatory activity of MIF *in vitro* or *in vivo*, and inhibition of morbidity or mortality in any of a number of animal models of human diseases that are characterized by the release, production and/or appearance of MIF. The activity of MIF

20 antagonists may also be assessed in various well-known and accepted *in vitro and in vivo* models of cancer, including particularly tumor cell proliferation assays, tumor cell killing assays, solid tumor growth and/or metastasis assays, and in various known and accepted *in vitro and in vivo* models of angiogenesis or neovascularization including, for example, endothelial cell proliferation and differentialtion assays, Matrigel™ assays, angiogenesis and

25 tumor angiogenesis assays, and the like.

Compounds **10** (corresponding to L-TrpME/parahydroxybenzaldehyde Schiff base) and **9** (corresponding to L-TyrME/parahydroxybenzaldehyde Schiff base) exhibited MIF inhibitory activity in a representative assay for MIF biological activity (*i.e.*, inhibition of MIF induced

30 proliferation in quiescent NIH/3T3 cell cultures) and inhibited enzymatic activity with IC_{50} values of about 5 μM and 10 μM, respectively. Neither compound **8** (corresponding to L-PheME/parahydroxybenzaldehyde Schiff base) nor compound **10b** (corresponding to D-TrpME/parahydroxybenzaldehyde Schiff base) exhibited anti-MIF biological activity (NIH/3T3 cell proliferation assay) and these compounds exhibited IC_{50} values for MIF

35 tautomerase inhibition of about 50 μM and 100 μM, respectively. The activity of compound **10** is further exemplified by reference to Figure 1, which summarizes experimental results showing that compound **10** is as effective an MIF antagonist as a neutralizing anti-MIF monoclonal antibody (mAb). In the illustrative experiments, briefly, DME medium (supplemented with 10% serum) was added to quiescent NIH/3T3 fibroblasts together with a

26

neutralizing anti-MIF mAb, or L-tryptophan/para-hydroxybenzaldehyde Schiff base (i.e..
compound 10) or D-tryptophan/para-hydroxybenzaldehyde Schiff base (i.e., compound 10b).
Proliferation was assessed by the incorporation of [3H]-thymidine into DNA and %
suppression of proliferation was calculated. The results shown are the mean of duplicate
5   assays and are representative of two separate experiments. Therefore, these data show a
correlation between inhibition of MIF tautomerase activity and anti-MIF biological activity.

The activity of compound 10 as an MIF antagonist is still further exemplified by
reference to Figure 2, which summarizes experimental results showing that compound 10 is as
effective an MIF antagonist agent as is a neutralizing anti-MIF monoclonal antibody (mAb).
10   The following table correlates the experimental conditions with the bar graph shown in Fig. 2.

| Bar #	Experimental condition			
1	+ serum			
2	+ serum-free			
3	+ serum-free	+ rMIF	+ L-trp/SB at 10 μM	(compound 10)
4	+ serum-free	+ rMIF	+ L-trp/SB at 1 μM	(compound 10)
5	+ serum-free	+ rMIF	+ D-trp/SB at 10 μM	(compound 10b)
6	+ serum-free	+ rMIF		

20   In the illustrative experiments. cultured embryonic fibroblasts are induced to enter
apoptosis (cell death) as a result of serum withdrawal. These cultures can be "rescued" by
treatment with MIF. Thus, test compounds are conveniently assayed for MIF antagonist
activity by reference to the activity of the compounds to neutralize the effect of added MIF in
the apoptosis assay: that is, by the persistence of apoptosis (cell death) in serum-free culture
25   conditions despite the addition of MIF. In this regard, anti-MIF antibodies are known to
antagonize MIF and serve as a convenient positive control.

Briefly, primary murine embryonic fibroblasts are distributed into 6-well cell culture
plates at 2×10^5 cells/well. Cells are subsequently cultured overnight under standard
conditions in 10% serum-containing medium (+serum) or in serum-free medium (+serum-free)
30   in the absence or presence of recombinant human MIF (rMIF) at 50 ng/ml. Test compound
vehicle alone (Me₂SO) or Schiff base-type MIF antagonist test compounds (L-trp/SB = L-
tryptophan/para-hydroxybenzaldehyde Schiff base, compound 10: D-trp/SB = D-
tryptophan/para-hydroxybenzaldehyde Schiff base, compound 10b) dissolved in vehicle are
preincubated with rMIF in serum-free medium for five minutes prior to addition to cell
35   cultures. Cell lysates are analyzed for apoptosis by any of a variety of convenient methods,
such as ELISA for fragmented DNA. Results shown are the mean +/- SD of duplicate samples
and are representative of replicated experiments. At a dose of 10 μM, compound 10
significantly antagonizes the bioactivity of MIF in this in vitro assay that is predictive of
clinical utility for conditions, diseases, or indications wherein MIF is released from cellular
40   stores and/or MIF synthesis or appearance is increased.

We claim:

1. A compound for inhibiting MIF activity comprising a compound of Formula I:

5
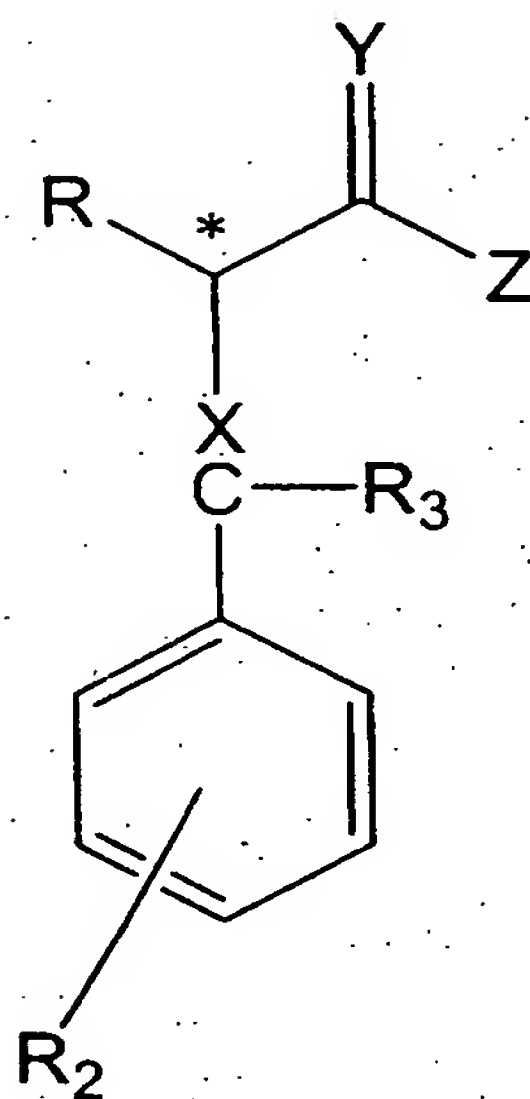
10   wherein X is nitrogen or carbon;

wherein Y is O, $C(R_1)_2$, or S;

wherein Z is OH, R_1, $-CH_2-N(R_1)_2$, or SR_1;

wherein R_1 is independently H, straight or branched C_1-C_6 alkyl, straight or branched C_2-C_6 alkenyl, or if R_1 is attached to carbon (but not if R_1 is attached to N or S), straight or

15 branched C_1-C_6 alkoxy;

wherein R_2 is a single or multiple substitution independently H, OH, R_1, $N(R_1)_2$, SR_1 or a halogen;

wherein R_3 is absent, H, R_1, or a halogen;

wherein * and C mark potentially asymmetric carbon atoms and, in each instance of R,

20 X, Y, Z, R_1, and R_3 where * or C or both are asymmetric, all diastereomers are included in Formula I;

wherein R is the side-chain of any naturally occurring alpha amino acid such that R together with X, Y and Z comprise, when Y is O and Z is OH, an aromatic, aliphatic or heterocyclic D or L alpha amino acid moiety linked as a Schiff base or a reduced Schiff base

25 (if X is N) or as a carba analog thereof (if X is C) to an optionally substituted benzaldehyde moiety, and wherein if Y is other than O, or Z is other than OH, R together with X, Y and Z

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comprise a substituted amino acid moiety, an analog of an amino acid moiety or a protected amino acid moiety;

and pharmaceutically acceptable salts thereof.

2.   The compound of claim 1 wherein R is a side chain of any naturally-occurring alpha amino acid.
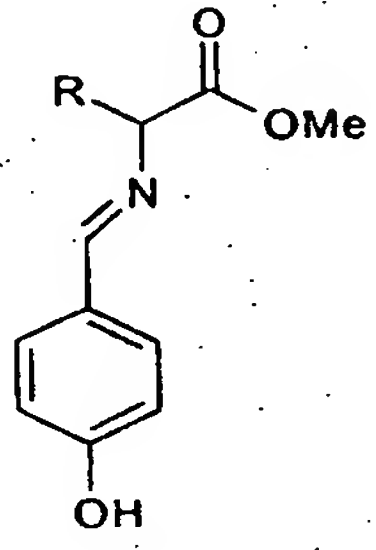
3.   The compound of claim 1 wherein X is N, Y is O, and Z is OMe such that together with R they form a protected L aliphatic or aromatic amino acid moiety.

4.   The compound of claim 1 wherein R_2 is OH, and R_3 is H.

5.   The compound of claim 4 wherein R_2 is in a para position to comprise a para-hydroxybenzaldehyde moiety.

6.   The compound of claim 1 wherein the compound is selected from Formula II wherein R is H (compound 1); wherein R is -CH_3 (compound 2 or compound 2a corresponding to L alanine and compound 2b corresponding to D alanine); wherein R is -$CH(CH_3)_2$ (compound 3); wherein R is -$CH_2CH(CH_3)_2$ (compound 4); wherein R is -$CH(CH_3)CH_2CH_3$ (compound 5); wherein R is -$CH_2CH_2SCH_3$ (compound 6); and wherein R is -CH_2OH (compound 7)

Formula II:

R		Inhibition (IC_{50})
R=H	1	>100μM
R= CH_3	2a	80μM
R= CH_3	2b	70μM
R= $CH(CH_3)_2$	3	>100μM
R=$CH_2CH(CH_3)_2$	4	
R=$CH(CH_3)CH_2CH_3$	5	
R=$CH_2CH_2SCH_3$	6	100μM
R=CH_2OH	7	100μM

7.   The compound of claim 1 wherein R is the side-chain of an aromatic alpha amino acid, X is N, Y is O, and Z is OMe such that together with R they form a protected L aromatic amino acid moiety.

8.   The compound of claim 7 wherein R_2 is OH, and R_3 is H.

9.   The compound of claim 7 wherein R is -CH_2-phenyl (compound 8), or R is -CH_2-para-hydroxyphenyl (compound 9), or R is -CH_2-indoyl (compound 10).

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10.   A pharmaceutical composition comprising a compound from claim 1 or a pharmaceutically acceptable salt thereof, together with a pharmaceutically acceptable carrier or diluant.

11.   A compound of Formula I, or a pharmaceutically acceptable salt thereof, as a
5   pharmaceutical composition comprising either of the aforesaid, for use in a medicine or for the manufacture of a medicament for the treatment or prevention of cancer or of inflammatory disorders including arthritis, proliferative vascular disease, ARDS (acute respiratory distress syndrome), cytokine-mediated toxicity, sepsis, septic shock, psoriasis, interleukin-2 toxicity, asthma and MIF-mediated conditions.

10   12.   A method for treating or preventing cancer or immune, autoimmune, or inflammatory disorders including arthritis, proliferative vascular disease, ARDS (acute respiratory distress syndrome), cytokine-mediated toxicity, sepsis, septic shock, psoriasis, interleukin-2 toxicity, asthma, MIF-mediated conditions, rheumatoid arthritis, insulin-dependent diabetes, multiple sclerosis, graft versus host disease, lupus syndromes, and
15   conditions characterized by local or systemic MIF release or synthesis, comprising administering an effective amount of a compound or combination of compounds of Formula I.

13.   A method for treating or preventing a disorder or disease condition characterized by undesired MIF activity comprising administration of a compound of Formula I in conjunction with at least one steroidal or non-steroidal anti-inflammatory agent.
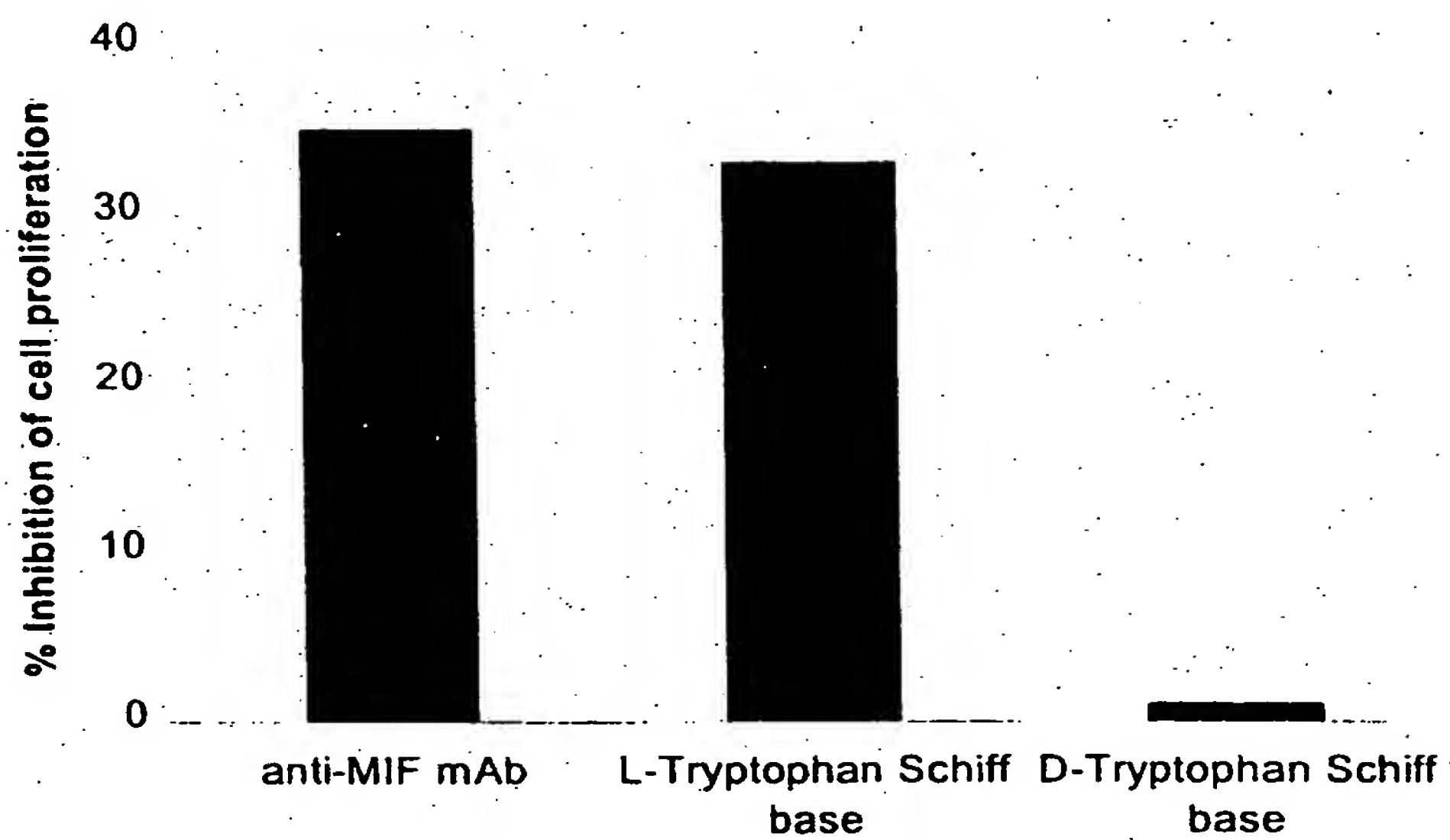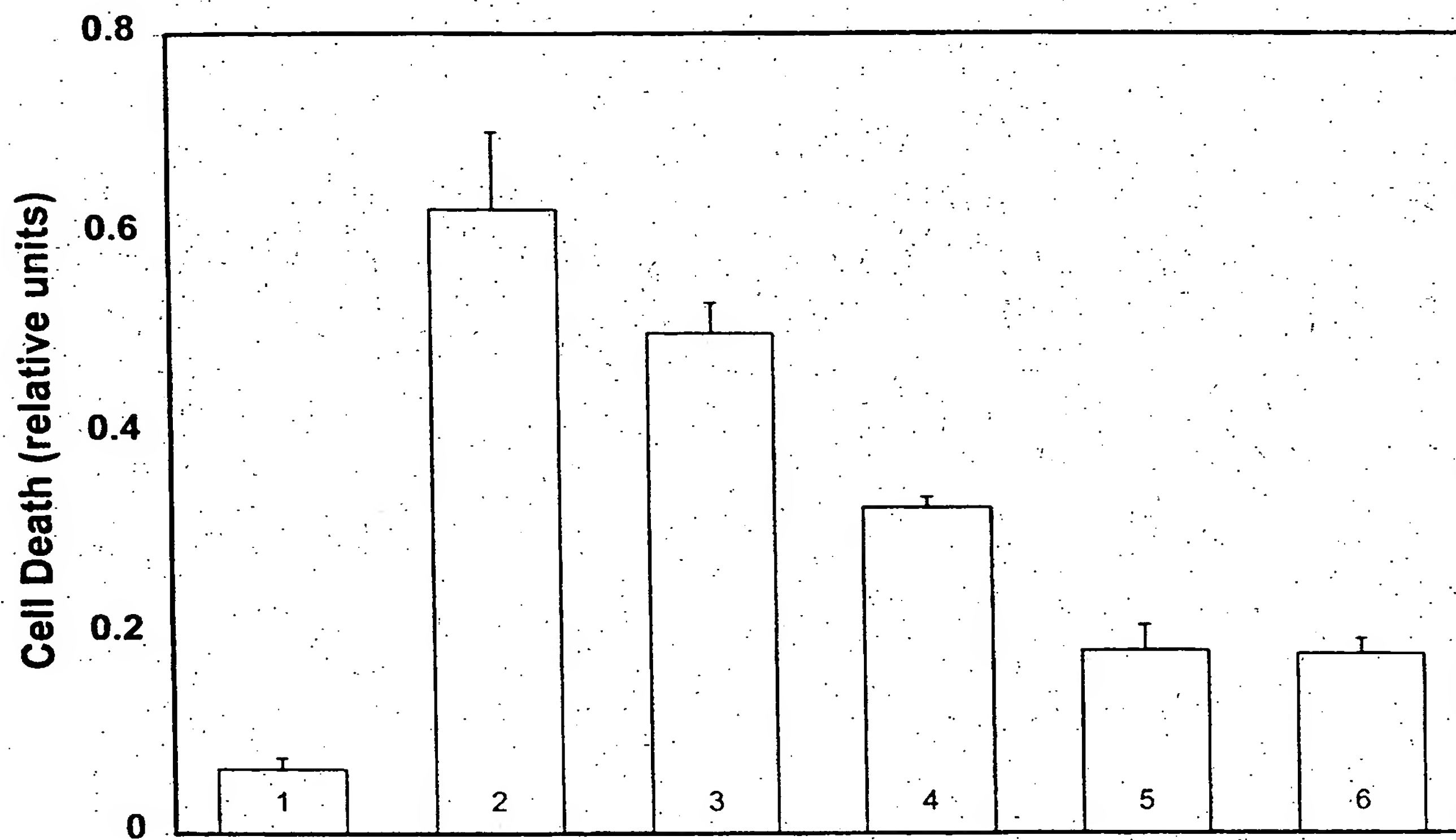
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Figure 1

Figure 2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/29554

A.   CLASSIFICATION OF SUBJECT MATTER

IPC(7)   :   C07C 249/00, C07C 229/00, C07C 211/00, C07F 9/02
US CL   :   562/440, 560/035, 564/336, 558/231

According to International Patent Classification (IPC) or to both national classification and IPC

B.   FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
    U.S. : 562/440, 560/035, 564/336, 558/231

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN Database: Formula I compound having anti-cancer, anti arthritis or anti-asthma effect searched

C.   DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Databse USPATFULL on STN (Columbus, OH, USA), No.131:271690; Synthesis of 2',5' - dihydropyrrolo[3',4':1,2][60]fullerene derivatives,abstract,Wu et al., May 1999.	1-12

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

<table>
<tr><td colspan="2">Special categories of cited documents:</td><td>"T"</td><td>later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td></tr>
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<tr><td>"E"</td><td>earlier application or patent published on or after the international filing date</td><td>"X"</td><td>document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td></tr>
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<tr><td>"P"</td><td>document published prior to the international filing date but later than the priority date claimed</td><td>"&"</td><td>document member of the same patent family</td></tr>
</table>

Date of the actual completion of the international search	Date of mailing of the international search report
12/22/00	**12 APR 2001**

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Gary Geist *[signature]* Telephone No. 703-308-0193

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